MOLECULAR HELMINTHOLOGY: AN INTEGRATED APPROACH

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Structure and Function of Helminth Surfaces-I

I 001 CELL BIOLOGY OF THE SCHISTOSOME SURFACE, John P. Caulfield, Syntex Discovery Research, Palo Alto, CA

Schistosomes live in the portal blood vessels of their vertebrate hosts for years. The surface of the parasite that interfaces with the host is a syncytium. The following summarizes cell biological data on this organ:

Morphology: The syncytium consists of 3 major elements: 1) The tegument, a 1-2 µm thick cytoplasm-like structure containing contractile proteins and a few mitochondria, covers the parasite and is convoluted into pits and spines. The tegument is covered by two closely opposed lipid bilayers. Only the inner of the bilayers i. e., the bilayer facing the parasite, appears to be a true membrane. 2) Internuncial processes connect the tegument to 3) cytons that lie deep within the worm and contain nuclei and synthetic machinery.

Chemistry: The small numbers of worms that can be obtained and the presence of host molecules on their surface compromises the chemical definition of the bilayers. The major lipids appear to be phosphatidylcholine, phosphatidylethanolamine, and cholesterol. Most proteins on the parasite surface are anchored by GPI and photobleaching measurements suggest there are few transmembrane proteins in the outer bilayer. However, one membrane spanning protein has been found. A variety of enzymes are associated with the tegumental membranes but their disposition within the bilayers is unknown.

Turnover/Function: In vitro measurements with exogenously delivered probes suggest that the outer bilayer is shed into the medium both rapidly, following exponential decay kinetics with a $t_{1/2}$ = 12 hr, and slowly, on the order of days. Lipids are probably responsible for the faster rates but the slower rates may be artifactual, arising through cross-linking of probes and ligands external to the bilayer. Alternatively, both rates may obtain in vivo with the slower rate serving to conserve membrane proteins. It is not clear whether the parasite can regulate membrane turnover. Further, although transport has been described across the tegument, the molecular arrangement of transporters in the two bilayers is unknown. Multivalent ligands bound to the surface of the parasite are not endocytosed, suggesting that the outer bilayer is not linked to the cytoskeleton. Perhaps, the function of the outer bilayer is to mask the worm from the host immune system and to deflect toxic or lytic host molecules from entering the inner bilayer. Further, the two bilayers may produce monopalmitylysophosphatidylcholine that lyses cells adherent to the parasite.

Biogenesis: The lipids for the bilayers are derived from the host since the parasite cannot synthesize either sterol or fatty acids and does not modify phospholipid head groups. Amino acids and lipids are most likely acquired through the breakdown of host blood cells, lipoproteins, and serum proteins in the gut, transported across the gut epithelium and ultimately into the cytons, assembled into bilayers, and transported to the surface via membrane bound organelles called multilamellar bodies. An alternative pathway of transport across the tegument may also exist. The high rates of turnover, about 10^3 µm²/hr, are apparently accomplished with minimal energy consumption.

Model: The outer bilayer is most probably a simple lipid bilayer that contains few proteins and protects the inner bilayer which functions similarly to a mammalian plasma membrane. The major function of the outer bilayer appears to be defense against both recognition and attack by the immune system. The outer bilayer must be semipermeable, however, to permit water soluble molecules to reach the inner bilayer for transport and to account for the low electrical resistance measured across the tegumental membranes. Openings in the outer membrane could occur either in discrete sites such as the pits or in submicroscopic pores.

I 002 THE ROLE OF TYROSINE DERIVED CROSS-LINKS IN THE CUTICULAR BIOCHEMISTRY OF PARASITIC NEMATODES, Raymond H. Fetterer and Marcia L. Rhoads, Helminthic Disease Laboratory, Livestock and Poultry Sciences Institute, United States Department of Agriculture, Beltsville, Maryland 20705.

The cuticle is a major interface between host and parasite and plays a central role in nematode biology. Interference with cuticular synthesis or function may provide the basis for development of novel controls of livestock parasitic nematodes. The cuticle consist of a thin, flexible outer covering and is composed of collagen-like proteins that constitute the medial and basal layers and non-collagen proteins forming the epicuticular and external cortical regions. Studies were initiated to determine the occurrence and synthesis of the cross-linking amino acids dityrosine and isotrityrosine in the cuticular proteins of Ascaris suum and Haemonchus contortus. Acid hydrolysates of collagenous and noncollagenous cuticular proteins were analyzed by high performance liquid chromatography (HPLC) for tyrosine derived cross-linking amino acids. Dityrosine and isotrityrosine were identified by their chromatographic behavior, absorbance spectra and other chemical characteristics and were found in collagenous and noncollagenous proteins from adult and parasitic larval stages. Synthesis of cross-links was investigated by measuring the incorporation of radiolabeled tyrosine precursor into dityrosine and isotrityrosine in A. suum larvae. Tritiated dityrosine and isotrityrosine were detected by HPLC analysis of cuticular proteins after 96 hr incubation in [^{3}H]tyrosine. Sixty percent of the HPLC recovered radiolabel was present as tyrosine, 20% as dityrosine and 6% as isotrityrosine. A similar distribution of radioactivity was observed in cuticular proteins from young adults of A. suum following 48 hr in vitro incubation with [^{3}H]tyrosine. The noncollagenous cuticular protein from the larval stages had higher rate of synthesis of [^{3}H]dityrosine than did the collagenous cuticular proteins, whereas the collagenous cuticular proteins had higher rates of synthesis of [^{3}H]isotrityrosine. Pulse-chase studies demonstrated a relatively low rate of synthesis of both dityrosine and isotrityrosine and the addition of the putative peroxidase inhibitors, phenylhydrazine (PHEN), 3-amino-1,2,4, aminotriazole (AT) and N-acetyltyrosine (NAT) to the culture media reduced the amount of [^{3}H]tyrosine synthesized into both dityrosine and isotrityrosine. Cell-free extracts of A. suum larvae also converted radiolabeled tyrosine to dityrosine but isotrityrosine was produced by only some extracts. The rate of conversion was inhibited by PHEN, and to a lesser degree by AT and NAT. These results suggest that the tyrosine residues of cuticular proteins are posttranslationally modified by the formation of dityrosine and isotrityrosine cross-links, and this modification is most likely mediated by a peroxidase.

I 003 FORMS AND FUNCTIONS OF NEMATODE SURFACES, Rick Maizels, Mark Blaxter, Edith Cookson, David Gems, Tony Page, Murray Selkirk, Wellcome Research Centre for Parastic Infections, Imperial College of Science, Technology and Medicine, London UK.

Parasitic and free-living nematodes show a diversity of surface structures at both the cuticular and extracuticular levels. The structural components of the cuticle (collagens, cuticlin) are well conserved, but species and life cycle stages differ in the repertoire of membrane-associated and soluble proteins expressed. Several such proteins have been characterised and cloned in our studies on *Brugia malayi* adult parasites and *Toxocara canis* infective larvae. The major soluble surface protein of *B.malayi*, gp29, encodes a homolog of glutathione peroxidase [1] while the most prominent integral cuticle protein of *T.canis* (TES-32) has strong homologies with mammalian lipid binding proteins. Each of these products are conserved between related nematode species inhabiting similar niches. The functional implications of these will be discussed in the context of nematode biology.

Extracuticular structures are represented by surface coats, generally of a polyanionic glycocalyx nature [2]. Using *T.canis* as a model system, we have shown that the synthesis and secretion of the surface coat can be traced to the esophageal and secretory glands, ducted to the cuticle [2]. The major coat component is a heavily O-glyosylated complex which migrates around 120 kDa on SDS-PAGE; evidence now suggests that the core protein for this complex is a serine/threonine-rich 15 kDa product with mucin-like characteristics which is represented in larvae by a hyper-abundant mRNA. The O-linked glycans are known to be principally a blood-group like trisaccharide with methylation modifications [3]. The coat therefore may act as a lubricant and facilitator of migration through the tissues. The surface coat confers additional properties on the nematode surface, the most dramatic of which is the ability to slough off antibody and granulocyte attack [4]. Thus nematode surfaces contain novel sets of protein and carbohydrate molecules arraigned at different levels to mediate various functions essential to survival in the mammalian host.

[1] Cookson E, Blaxter ML & Selkirk ME (1992) Proc Natl Acad Sci USA. 89: 5837-5841

[2] Blaxter ML, Page AP, Rudin W & Maizels RM. 1992. Parasitol Today 8: 243-247.

[3] Page AP, Hamilton AJ & Maizels RM. 1992. Exp Parasitol 75: 56-71.

[4] Khoo K-H, Maizels RM, Page AP, Taylor GW, Rendell N & Dell A. 1991. Glycobiology 1: 163-171.

[5] Page AP, Rudin W, Fluri E, Blaxter ML & Maizels RM. 1992. Exp. Parasitol. 75: 72-86.

I 004 GENETIC ANALYSIS OF SURFACE COMPOSITION IN CAENORHABDITIS ELEGANS, Samuel M. Politz, David G. Grenache, and Shellee Morehead, Worcester Polytechnic Institute, Worcester, MA 01609.

C. elegans genes that affect cuticle surface composition (termed srf genes) have been identified by formal genetic analysis of mutations in conjunction with indirect immunofluorescence experiments, using antibody probes directed against surface antigen markers. Two phenotypically distinct srf mutant classes, defective srf mutants (srf-d mutants) and heterochronic srf mutants, show alterations in the expression of surface antigen markers that appear in wild-type only on the surface of the first (L1) larval stage. The availability of monoclonal antibody probes for these L1-specific markers has allowed biochemical characterization of the surface molecules as well as genetic and phenotypic analysis of marker expression in live animals.

Mutations in srf-d genes srf-2 and srf-3 appear to cause loss of expression of surface antigen markers including the L1-specific markers recognized by monoclonal antibodies M37 and M38. L1s of these mutant strains do not bind these monoclonal antibodies. Complementary immunofluorescence experiments using polyclonal anti-cuticle antisera suggested that the loss of surface determinants in srf-2 and srf-3 mutants causes unmasking of antigenic determinants that are normally hidden in the wild-type cuticle. The fact that all available srf-2 and srf-3 mutants have recessive phenotypes further suggests that these mutations cause loss of function in genes involved in specific steps of surface molecule biosynthesis, assembly, or transport. Partial biochemical characterization of the antigen recognized by MAb M38 in gel immunoblotting experiments indicated that the expression of a cuticle-associated O-linked glycoprotein is defective in srf-2 and srf-3 mutants, raising the possibility that the mutant lesions may be in post-translational modification steps.

In contrast to the srf-d phenotypes, mutations in the heterochronic srf gene srf-6 cause the L1-specific markers recognized by MAb M37 and M38 to appear on the surface of temporally inappropriate stages L2-L4 in addition to the L1 stage. srf-6 mutants show no other obvious phenotype besides this alteration in timing of surface antigen expression. However, the srf heterochronic phenotype is also exhibited by strains carrying mutations in previously characterized ts dauer-constitutive genes. These daf-c mutants grow normally at 16° but form dauer larvae at 25°. When grown at 16°, strains carrying mutations in six of seven daf-c genes tested showed binding of MAb M37 at stages L1-L4, just as the srf-6 mutants did. The daf-c genes appear to be involved in switching on C. elegans dauer larva formation in response to specific environmental signals. The present evidence suggests that some of these same genes may also be involved in stage-specific surface antigen switching in C. elegans.

Structure and Function of Helminth Surfaces-II

I 005 **NEUROBIOLOGY AND GENETICS OF FEEDING IN *CAENORHABDITIS ELEGANS*, Leon Avery, Harry Duttweiler, Raymond Lee, David Raizen, and Hans Rosenfeldt, Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235-9038.**

The pharynx, a nearly self-contained neuromuscular organ containing 80 nuclei, is responsible for feeding. Twenty pharyngeal neurons constitute the pharyngeal nervous system. Functional pumping continues when all 20 pharyngeal neurons are killed, showing that the muscles know how to execute this motion without detailed direction from the nervous system. The pumping that occurs in the absence of the pharyngeal nervous system is however severely abnormal, showing that the pharyngeal nervous system has a regulatory role. The pharyngeal nervous system regulates pumping in (at least) two ways. First, it regulates the rate of pumping. Second, it regulates the timing of pharyngeal muscle motions within a pump.

Pumping rate increases in the presence of food. A pair of pharyngeal sensory neurons called the MCs are necessary for most of this increase. In the absence of food, pumping continues at a low rate. This basal pumping is affected by the neurotransmitter serotonin, probably from the extrapharyngeal neuron RIH. RIH is connected indirectly to the pharyngeal I1 neurons, which have synapses to the MCs and are necessary for normal rates of basal pumping and for imipramine stimulation of basal pumping. Serotonin can however stimulate pumping even when the I1 neurons have been killed, suggesting that, in addition to the RIH→→I1→MC pathway, there may be a second pathway in which serotonin from a source other than RIH (the most plausible is the pharyngeal NSM neurons) acts directly on the MCs or pharyngeal muscle. We suggest the purpose of basal pumping regulated by the I1 pathway is to sample the environment, bringing bacteria into the pharynx where they can activate pharyngeal sensory neurons MC and possibly NSM, which then cause rapid pumping.

The pharyngeal nervous system also regulates the timing of pharyngeal muscle relaxation, and this regulation is important for effective trapping and transport of food within the pharyngeal lumen. The sensorimotor neuron M3 acts to speed up pharyngeal relaxation, and is inhibited by the sensory neuron I5.

Mutants with defective feeding behavior are easily isolated by screening the F2 self-progeny of mutagenized hermaphrodites for worms with starved appearance and visibly abnormal feeding behavior. Using this screen we identified 52 mutations in 35 genes that affect feeding behavior. Mutants defective in genes in the largest class (23 genes) have phenotypes reminiscent of worms with damaged pharyngeal nervous systems, suggesting that these genes are necessary for pharyngeal nervous system function. Mutations in one gene, *eat-5*, disrupt the synchronization of pharyngeal muscles.

Four more genes may be members of a family involved in muscarinic control of pharyngeal relaxation. Mutations in *eat-12* and *eat-6* block or delay relaxation of pharyngeal muscles. Indirect evidence suggests that these mutations block or delay return of muscle membrane potential to resting levels. Muscarinic acetylcholine agonists produce a phenotype similar to that of *eat-12* and *eat-6* mutants. A mutation in the gene *eat-11* causes hypersensitivity to the acetylcholine agonist arecoline. Gain-of-function mutations in the gene *egl-30* suppress *eat-11* (both the arecoline hypersensitivity and the visible phenotypes).

I 006 COLLAGEN GENES IN *C. ELEGANS*, James M. Kramer, Adam Levy, Jie Yang, Marion Sibley, Malini Gupta, Patricia Graham, and Michael Schwen, Northwestern University Medical School, Dept. of Cell, Molecular and Structural Biology, Chicago, IL 60611.

The genome of *C. elegans* contains between 50 and 150 collagen genes. Two of these genes encode basement membrane-specific type IV collagen chains, while most, or all, of the remainder encode components of the cuticle. The cuticle collagens have a stereotypical domain structure that is conserved amongst all nematode species examined. They all have two cysteine residues located just amino and carboxyl to the Gly-X-Y repeat domains, and either two or three cysteines in the major interruption of the repeats. Four short stretches of amino acid sequence conservation, termed Homology Blocks A-D, are also found in the amino non-(Gly-X-Y) domains of most of these collagens. Mutations in several cuticle collagen genes (*dpy-2, dpy-10, dpy-13, rol-6, sqt-1*) have been shown to cause alterations in the organism's morphology. Different mutations can produce animals with long (Lon), short and fat (Dpy), right roller (RRol), or left roller (LRol) morphologies. A dominant **RRol** phenotype results when either of two conserved arginine residues in Homology Block A (HBA; consensus RxxRQAY) is changed to cysteine in *rol-6* or *sqt-1*. The equivalent arginine to cysteine change in *dpy-10* produces a dominant **LRol** phenotype, suggesting that *dpy-10* functions in a mirror image manner relative to the *rol-6* and *sqt-1* collagens. A recessive LRol phenotype results when one of the conserved cysteines carboxyl to the Gly-X-Y repeats of *sqt-1* is changed to either serine or tyrosine. Thus, removing a cysteine from the carboxyl end of *sqt-1* produces the opposite phenotype that adding a cysteine towards the amino end of the molecule does. The null phenotypes for both *rol-6* and *sqt-1* are nearly wild-type, while the *dpy-10* null phenotype is dumpy left roller. Replacement of glycines in the Gly-X-Y repeats of *sqt-1* cause mild, non-temperature sensitive phenotypes, while similar mutations in *dpy-2* and *dpy-10* cause severe phenotypes that are often ts. The contrasts between *dpy-2/dpy-10* and *rol-6/sqt-1* demonstrate the distinct roles that different collagens can have in normal cuticle function. Utilizing *in vitro* mutagenesis and transgenic analysis, HBA has been shown to most likely function as a protease cleavage site. Replacement of arginine with lysine gives wild-type function, histidine produces a recessive RRol phenotype, and other tested replacements result in a dominant RRol phenotype. Histidine may allow partial cleavage at HBA to result in the recessive phenotype. Thus, the dominance of the arginine to cysteine mutations is due to loss of the arginine, not the presence of a novel cysteine.

Mutations in the two genes (*emb-9* and *let-2*) that encode the basement membrane-specific (type IV) collagen chains in *C. elegans* can cause embryonic lethality, demonstrating the importance of these extracellular matrix molecules in embryonic development. Most of these mutations are replacements of glycines in the Gly-X-Y repeats, and presumably interfere with triple-helix assembly. The *let-2* mRNA, which encodes the α2(IV) chain, is alternatively spliced, with one form being predominant in embryos and the other predominant in larvae and adults. Form-specific antisera have been used to show that the two alternative forms of the type IV collagen correspond to differences in the crosslinking between basement membrane components. These results indicate that there may be fundamental differences in the properties of embryonic and post-embryonic basement membranes in *C.elegans*.

I 007 MOLECULAR BIOLOGY OF CUTICULAR AND RELEASED PROTEINS OF FILARIAL PARASITES, Larry A. McReynolds[1] Catherine B. Poole[1], Yan Hong[1], Claude V. Maina[1] and Murray E. Selkirk[2], [1]New England Biolabs, Beverly, MA and [2]Imperial College, London, United Kingdom.

Excretory/secretory and surface proteins often produce a significant immune response from the the host. In order to understand the role of filarial proteins in host immunity and pathology we have cloned and characterized antigens using selected antisera. Dogs were immunized against the filarial parasite Dirofilariae immitis (dog heart worm) with irradiated L3s. Antisera from these animals was used to screen a lambda gt11 cDNA library. One of the major immunogens, called Di5, was determined by DNA sequence analysis to be a 399 base pair repeat. A Western blot, using specific antisera generated to the cloned Di5 protein, showed a striking ladder-like pattern in extracts of D. immitis adults. The native protein has over 20 similar repeats (16kD) that are organized in a tandem array. Pulse chase studies indicate that it is synthesized as a very high molecular wieght precursor, over 200kD and then cleaved to generate the ladder-like array. Protein sequence of the amino terminus of the protein demonstrated that cleavage took place just prior to four basic amino acids. A search for the parasite protease responsible for the cleavage is now underway.

The Di5 monomer is the major excretory/secretory protein of adult male and female D. immitis. The Di5 protein is antigenic in immune and infected dogs. Anti-Di4 IgG antibody titers increase in D. immitis infected dogs as immature adult parasites reach the heart. DNA hybridization studies and sequence comparisons demonstrate that this protein is present in other parasitic nematodes including those that infect man: Brugia malayi, Brugia pahangi, Loa loa and Ascaris suum. A vaccine trial in dogs using the RIBI adjuvant resulted in a high antibody response to the protein but did not confir protection.

A surface localized D. immitis antigen that we have recently characterized has sequence homology to the cystein protease inhibitor cystatin. The recombinant proteins inhibits papain with a Ki of 13 nM. The mRNA for the protein has a spliced leader inserted at two different sites in the initial transcript. Both transcripts produce the same protein. The target for the parasite cystatin, a host or parasite protease, and a possible role in immunity is currently being studied.

I 008 PATTERN AND PERIODICITY OF DEFECATION IN *C. elegans*, James H. Thomas, Dennis W.C. Liu, and David J. Reiner, Department of Genetics, University of Washington, Seattle.

C. elegans defecates by periodically activating a stereotyped motor program. Each defecation consists of the action of three distinct sets of muscles in a coordinated pattern. pBoc, aBoc, and Exp are acronyms for these three muscle movements. The normal motor program consists of pBoc followed about four seconds later by a nearly simultaneous aBoc and Exp. In the presence of bacteria at 25° this program repeats regularly every 45 seconds. A variety of behavioral analysis suggests that this periodicity is under the control of a neural clock. This periodically activated motor program is well suited to genetic analysis because it occurs regularly and frequently and many defects in the motor program cause the easily scored phenotype of constipation (Con). My lab has identified about 25 genes that control this motor program by screening either for Con mutants or by direct observation of the motor program. The primary findings thus far answer some genetics-style questions about the control of complex behavioral patterns. Such patterns have been previously studied only from behavioral or physiological angles. Nearly all of the genes identified fall into two general categories: a set of about 20 genes that control specific parts of the motor program, and a set of at least five genes that control the periodicity of the motor program. The first set of genes themselves fall into several major groups. Mutations in seven genes specifically eliminate the Exp motor step without affecting the other motor steps or the cycle period. Similarly, mutations in two genes affect only pBoc, mutations in four genes specifically affect aBoc, and mutations in six genes affect aBoc and Exp but not pBoc. Many of these genes are defined by more than one recessive allele and their phenotypes may reflect their null phenotype. We conclude that each step in the motor program is at least partly under independent genetic control. The second set of genes includes at least five that specifically control timing of the motor program. Screens for such mutants are difficult and are not near saturation. A recessive mutation in one of these genes causes a short cycle period of about 25 seconds. A recessive mutation in another gene causes a split or echo cycle: a primary strong motor program is followed about ten seconds later by a second, often incomplete, motor program. Recessive mutations in at least three genes cause a long cycle period, the longest being over twice normal. All of the above mutants retain clear periodicity, although altered in length or pattern. Recessive mutations in at least one gene cause variable periodicity: individual worms alternate between bouts of very rapid cycling and bouts of no motor programs. These mutant classes indicate that the control of cycle periodicity and cycle period length are under genetic control independent of the execution of the motor program. Our studies thus far demonstrate that study of complex behavioral patterns is feasible at the genetic, and ultimately molecular, level.

Neurobiology of Helminths

I 009 SEROTONIN, SEROTONIN-DEFICIENT MUTANTS AND MALE MATING BEHAVIOR IN THE NEMATODE *C. ELEGANS*. Curtis M. Loer* and Cynthia J Kenyon. Department of Biochemistry & Biophysics, University of California, San Francisco. *present address - Department of Pathology, Laboratory of Molecular Pathology, 4150 Clement St. 113B, University of California, San Francisco, CA 94121.

Serotonin has been detected in a number of free-living and parasitic nematodes by immunocytological and chromatographic methods; it has been suggested to be involved in locomotion, reproductive behaviors, pharyngeal pumping, and even carbohydrate metabolism (Croll, Can. J. Zool. 53: 894, 1975; Donahue et al., Biochem. Biophys. Res. Comm. 101: 112, 1981; Horvitz et al., Science 216: 1012, 1982; Avery and Horvitz, J. Exp. Biol. 253: 263, 1990). In *Caenorhabditis elegans* as well as other nematodes, serotonergic fibers innervate vulval regions and serotonin can induce egg laying; serotonergic innervation in the male tail has suggested male-specific functions as well. In *C. elegans*, serotonin immunoreactivity is present in a number of identified neurons, including neurons known to be required for reproductive behaviors (Desai et al., Nature 336: 638, 1988; Chalfie and White, in *The Nematode Caenorhabditis elegans*, ed. W. B. Wood, p. 337, 1988). Our work shows that serotonergic neurons are important in male mating behavior and further suggests that serotonin or other biogenic amines are important not only for nervous function, but also for normal cuticle structure.

It is known that application of serotonin to male worms results in a tight ventral-inward curling of the tail, reminiscent of that exhibited during mating behavior. We have shown that laser ablation of male-specific serotonergic CP motoneurons results in males that are unable to execute this tail-curling behavior during mating. Mutations in the genes *bas-1*(biogenic amine synthesis abnormal), *cat-1*(catecholamine distribution abnormal), and *cat-4* result in worms in which serotonin immunoreactivity is reduced or absent. We have found that males with *bas-1*(kindly provided by G. Garriga), *cat-1*, and *cat-4* mutations display the same specific behavioral deficits as males in which CP neurons have been ablated. Isolation of mutants that alter these aspects of male mating behavior should yield additional serotonin-defective mutants, which may allow us to define how serotonin is made and used by *C. elegans*. We have found, for example, that the mutations *cat-1*, *cat-4* and *bas-1* are blocked at different steps in the serotonin synthetic pathway.

We have also found that worms carrying a mutation in the gene *cat-4*, previously shown to lack serotonin and dopamine (Desai et al., Nature 336: 638, 1988; Sulston et al., J. Comp. Neurol., 163: 215, 1975), also suffer from a serious cuticle defect, apparently making them more permeable than wild type worms to a variety of agents. The defects in *cat-4* mutants suggest that the nervous system and hypodermis underlying the cuticle share enzymatic or regulatory pathways for biogenic amines in *C. elegans*. Although nematode and insect cuticles are very different (collagenous vs. chitinous, respectively), it may be that biogenic amines are used by nematodes similarly to insects, that is, for cross-linking proteins in the cuticle (Wright, Adv Genet. 24: 127, 1987).

I 010 ELECTROPHYSIOLOGY OF *ASCARIS* MUSCLE, R.J. Martin, Department of Preclinical Veterinary Sciences, University of Edinburgh, Edinburgh EH9 1QH, U.K.

Each somatic muscle cell of *Ascaris suum* is divided into three regions: (i) The contractile spindle; (ii) The bag, an enlarged balloon-shaped structure which contains the nucleus and glycogen granules; (iii) The arm which is a process arising from the base of the bag and which joins the 'synctium', where it receives the synaptic input from the nerve cord.

The muscle resting potential is about -35mV and the membrane has been shown to have a high Cl permeability compared to Na and K. Superimposed on this potential are 3 types of depolarizing potential: (i) Ca spikes; (ii) Slow waves and (iii) Modulation waves. The depolarization of adjacent cells is correlated as a result of electrical coupling at the synctium; contraction is associated with the modulation waves. Ca currents and K currents responsible for the spikes can be seen under voltage-clamp. ACh depolarizes the muscle cells and opens non-selective cation channels located on the syncytial and bag membrane. The pharmacology of the ACh receptor is similar but not identical to the vertebrate ganglionic nicotinic receptor. GABA hyperpolarizes the muscle cells by opening Cl channels located at the syncytium and on the bag. The pharmacology of *Ascaris* GABA receptor differs from that of the vertebrate GABA receptors.

A muscle vesicle preparation has been developed which allows single-channel currents from the bag membrane to be recorded. ACh-activated channels show evidence of densensitization, two conductance levels (30-50pS and 15-25pS) and a mean open-time in the range 2-4ms.

The anthelmintics levamisole, pyrantel and morantel also activate the same channels. Their channel conductances are similar but they have shorter mean open-times. However these anthelmintics show differing degrees of open-channel block. GABA and piperazine open 20-40pS Cl channels which have mean open-times of 32 and 14ms respectively. Dihydroavermectin B1a does not activate GABA like channels, rather it acts as a non-competitive GABA antagonist. However it progressively opens 9-15pS Cl channels producing a 'staircase' effect. A Ca activated Cl channel is also found in the membrane and appears to underlie the resting membrane potential. It has a conductance of 200pS, is activated by Ca at the cytoplasmic membrane and is permeable to volatile fatty acids including alpha-methyl butyric acid. The volatile fatty acids are products of anaerobic breakdown of glycogen and their permeability suggests that this channel is involved in removal of anabolic wastes from the muscle cell.

These observations illustrate the value of the electrophysiological technique for investigating the physiology and pharmacology of nematode muscle and anthelmintic action (Martin et al, 1991).

Martin, R.J., Pennington, A.J., Duittoz, A.H., Robertson, S. and Kusel, J.R. (1991). The physiology and pharmacology of neuromuscular transmission in the nematode parasite *Ascaris suum*. Parasitology, 102, S41-S58.

I 011 **PHYSIOLOGY OF SCHISTOSOME MUSCLE CELLS, Ralph A. Pax and Tim A. Day, Michigan State University, East Lansing, MI 48824.**

Muscle fibers isolated from the platyhelminth Schistosoma mansoni have been studied with whole cell current- and volatge-clamp techniques. Fibers showed a marked time-dependent decrease in membrane resistance in response to depolarizing current injections. Voltage-clamp experiments revealed the presence of two distinct voltage-dependent outward currents. The most prominent current is a slowly activating, slowly and incompletely inactivating potassium current similar to delayed rectifier currents which have been described in a variety of cell types from a variety of organisms. Also present is a faster activating, quickly and completely inactivating potassium current that shares functional characteristics with "A"-currents. Three of the cell types studied possess a delayed rectifier current, but "A"-currents are present in only some of the fiber types. Though depolarization with high K^+ leads to contraction of the dispersed fibers, no voltage-dependent inward currents could be detected by whole cell voltage-clamp under any of our conditions.

Neurotransmitters and Neuropeptides in Helminths

I 012 NEUROPEPTIDES IN HELMINTHS, Chris Shaw[1] and David Halton[2], Comparative Neuroendocrinology Research Group, [1]School of Medicine, and [2]School of Biology and Biochemistry, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland, U.K.

With respect to organization of the nervous system, helminths are the first group in evolutionary terms to display cephalization of neural elements and a bilaterally-symmetrical neuronal plan. Indeed, recent models of metazoan evolution propose that all phyla displaying these features arose from a common flatworm-type ancestor. In terms of neuronal number and structural complexity the helminth nervous system is considerably simpler than that of more highly-evolved organisms. For this reason the nervous system of helminths could be regarded as a useful model in unravelling the intricate functions of the vertebrate homolog. However, the neurochemistry of helminth nervous systems now appears to be of a similar order of complexity to that of more highly-evolved organisms with well developed cholinergic, aminergic and peptidergic components. The latter component has been the major thrust of neurochemical research in our laboratory. The concept that neuropeptides due to their relative chemical complexity are more recent acquisitions in evolutionary terms than small and chemically-simpler classical neurotransmitters is probably no longer tenable or generally accepted. The plethora of immunocytochemical investigations in many helminth species would suggest that their nervous systems possess an array of neuropeptides. Such studies have been carried out using antisera generated to regulatory peptides from both higher invertebrate and vertebrate sources and these antisera usually recognize epitopes within their respective peptide antigens which are either bioactive sites and/or highly-conserved sequences. Phe-Met-Arg-Phe amide (FMRF amide) was originally isolated from a bivalve mollusc and antisera generated to this peptide immunostain large numbers of neurons and fibres in representative species from all major invertebrate phyla including many helminths. Similar if not identical immunostaining patterns have been obtained using antisera generated to the C-terminal hexapeptide amides of the much larger (36 amino acid residues) vertebrate peptides pancreatic polypeptide (PP) and neuropeptide Y (NPY). This was attributed in the past to antiserum cross-reactivity, as both classes of peptides terminate in a chemically-similar dipeptide (Arg-Phe amide and Arg-Tyr amide, respectively). Preliminary gel permeation chromatographic analysis of helminth extracts identified immunoreactivity coeluting with vertebrate PP/NPY and not with the much smaller FMRF amide. Radioimmunoassay with both PP and NPY hexapeptide antisera indicate that the helminth peptide was structurally more similar in its C-terminus to vertebrate PP than to NPY, and cross-reactivity studies indicated that the PP antiserum did not cross-react with FMRF amide even when present in a million-fold molar excess over PP. The cestode, *Moniezia expansa*, contained high concentrations of this PP immunoreactive peptide (192 ng/g) and was available in sufficient quantity to facilitate isolation. This was achieved by sequential reverse-phase HPLC and the purified peptide was subjected to structural analysis using mass spectroscopy and automated Edman degradation. These established a molecular mass of 4599 Da and the sequence of 30 residues. The discrepancy in these masses indicated the presence of additional residues which were subsequently identified by sequencing of endoproteinase Glu-C fragments. This PP immunoreactive peptide, designated neuropeptide F (NPF) consisted of 39 amino acid residues terminating in an Arg-Pro-Arg-Phe amide. Analogous neuropeptides have been isolated from a turbellarian (*Artioposthia triangulata*) and a mollusc (*Helix aspersa*) and have been detected in extracts of a coelenterate (*Hydra vulgaris*), three trematodes (*Schistosoma mansoni, Diclidophora merlangi, Fasciola hepatica*), two annelids (*Lumbricus terrestris, Nereis virens*) and several insects including the coackroach, *Periplaneta* and the fleshfly, *Sarcophaga*. NPF is thus an abundant neuropeptide with a widespread distribution in invertebrate nervous systems. These data have been substantiated in immunocytochemical and radioimmunometric studies using antisera specific for the C-terminal region of the molecule which exhibit negligible cross-reactivity with vertebrate PP/NPY and invertebrate FMRF amide. These studies authenticate the distribution of NPF and have established that FMRF amide immunocytochemistry of invertebrate nervous systems is largely erroneous in that FMRF amide antisera immunostain NPF. In conclusion, NPF is the most abundant neuropeptide so far detected in helminth nervous systems and indeed occurs in abundance in representatives of all major invertebrate phyla investigated. This peptide may be a generalized neurotransmitter within invertebrates and probably represents the phylogenetic precursor of vertebrate PP/NPY.

I 013 FMRFamide-LIKE NEUROPEPTIDES IN *CAENORHABDITIS ELEGANS*, Chris Li, Marc Rosoff, and Karin Schinkmann, Department of Biology, Boston University, Boston.

Neurotransmitter expression is critical for communication between cells of the nervous system. We have been investigating the developmental expression and regulation of the class of FMRFamide(Phe-Met-Arg-Phe-amide)-like neuropeptides in *C. elegans*. About 30 neurons, or roughly 10% of the neurons in *C. elegans*, stain with an anti-FMRFamide antiserum, and the onset of peptide expression appears to be developmentally regulated. The immunoreactive neurons include motoneurons, interneurons, and sensory neurons, as well as four gonadal cells. FMRFamide-like peptides can potentiate a serotonin effect in an egg-laying assay in *C. elegans*.

Seven putative FMRFamide-like peptides are encoded by two transcripts (transcripts A and B) of the *flp-1* gene in *C. elegans*; the *flp-1* encoded peptides all belong to the related class of FLRFamide-like peptides. The two transcripts arise by alternative splicing, resulting in a unique peptide encoded by only one of the transcripts (transcript B); the remaining encoded peptides are identical in transcripts A and B. Using reverse transcription/PCR, we have detected both transcripts in every developmental stage of the animal. Furthermore, quantitative PCR analysis of mixed stage reverse transcribed cDNA has revealed that transcript A is expressed at a two-fold higher level than transcript B. Genomic Southern analysis suggests that there is only one FMRFamide-like gene in *C. elegans*.

In collaboration with David Price, six of the seven FLRFamide-containing peptides predicted from the cDNA sequence have now been isolated from whole animal extracts by HPLC purification; these peptides represent the majority of immunoreactivity detected with our antiserum. The FLRFamide-containing peptide not isolated thus far is the unique peptide encoded only by transcript B; as transcript B is expressed at a lower level than transcript A, the unique peptide may be produced at a much lower concentration than the other peptides, and would, therefore, be more difficult to isolate. Two of the isolated peptides are identical to peptides isolated from the closely related nematode, *Panagrellus*. None of the FMRFamide-like peptides isolated from *Ascaris* has been found in *C. elegans*.

To examine the transcriptional regulation of the *flp-1* gene, we have used *lacZ* as a reporter gene under the transcriptional control of the *flp-1* promoter region for construction of transgenic animals. A promoter element that seems sufficient to elicit expression in specific cells in the head of the animal has been mapped to within 300 bp of the start site of transcription. Deletion analysis on this region is being performed to map further this element.

To position the *flp-1* gene on the physical and genetic maps, we probed an ordered array of overlapping YAC clones representing the entire worm genome (kindly provided by A. Coulson). Three overlapping clones hybridized to our probes, but the assignment of these clones on the physical map is ambiguous. To position *flp-1* on a linkage group, we are using the alternative strategy of RFLP analysis of the *flp-1* gene in Bristol and Bergerac strains of *C. elegans*. We have found a polymorphism in *flp-1* by Southern analysis of AseI digested genomic DNA from Bergerac and Bristol strains. This polymorphism is being used as a phenotypic marker to probe hybrid strains to determine the genetic map position of the *flp-1* gene.

I 014 THE CHARACTERISATION OF NEMATODE GABA RECEPTORS, David Laughton, Adrian Wolstenholme, Phillip Harris, Paul Towner, Muriel Amar and George Lunt, Department of Biochemistry, Bath University, Bath, U.K., BA2 7AY

The $GABA_A$ receptor of mammalian brain is a well-characterised member of the ligand gated ion channel receptor (LGICR) superfamily. The receptor is characterised by the presence of a number of separate but interacting binding sites for agents such as benzodiazepines, cage convulsants, picrotoxin, barbiturates and steroids. There is evidence that some of these sites are also present on the receptor complex from insects, namely the benzodiazepine, cage convulsant and picrotoxin sites. In nematodes there are reports of a $GABA_A$-like receptor on muscle cells of Ascaris suum but benzodiazepines, picrotoxin and cage convulsants are without effect. There are preliminary reports of the isolation of GABA receptor clones from C.elegans but no detailed pharmacological data have been presented. There have been reports that anti-rat $GABA_A$ R-antibodies cross-react with GABA receptors on C.elegans muscles and we have found evidence for similar cross-reactivity on pharyngeal gland cells and in gut. The effects of GABAergic ligands and some preliminary receptor binding studies led us to investigate the presence of $GABA_A$-like receptors in the parasitic nematode Haemonchus contortus. A molecular cloning approach has resulted in the isolation of at least two cDNAs that appear to be members of the LGICR family. One full length cDNA (HG10) shows approximately 35% identity with reported sequences for $GABA_A$ and Glycine receptor subunits. Expression of HG10 in Xenopus oocytes suggest that a functional homooligomeric receptor may be formed that is responsive to GABA and Glycine.

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I 015 AVERMECTIN RECEPTORS IN *C. ELEGANS*, James M. Schaeffer, Joseph P. Arena, Doris F. Cully, Edward C. Hayes, Ethel B. Jacobson, Susan P. Rohrer Elizabeth T. Birzin, Kenneth K. Liu, Easter G. Frazier, Philip S. Paress and Peter Meinke, Merck Research Laboratories, Rahway, NJ 07065

Avermectins are a family of macrocyclic lactones with potent insecticidal and nematocidal activity. Ivermectin, a semi-synthetic avermectin analog, is widely used in veterinary medicine as an anthelmintic and in humans to control *Onchocerca volvulus*, the causative agent of "river blindness". Our laboratory has used the free-living nematode *Caenorhabditis elegans* as a model system for characterizing the interaction between avermectin and its specific, high affinity binding site. I will describe two approaches taken by our laboratory for further characterization of this binding site; biochemical purification and expression of *C. elegans* mRNA in *Xenopus* oocytes.

Ivermectin binds with high affinity and in a stereoselective manner to membranes prepared from *C. elegans*. A large number of avermectin analogs have been evaluated in the binding assay and the strong correlation between binding affinity and nematocidal activity demonstrates the physiological importance of this binding site. Specific ivermectin binding sites were also identified in rat brain; however, the affinity was approximately 200-fold lower than that observed in *C. elegans* and stereospecificity studies demonstrated structural differences between the two binding sites.

A photoactive azido-avermectin analog has been synthesized and characterized. Azido-avermectin is biologically active and is a competitive inhibitor of ivermectin binding to *C. elegans* membranes. Radiolabeled azido-avermectin binds specifically to *C. elegans* membranes and upon photoactivation, covalently labels three polypeptides of 53, 47 and 8 kDa. The photoaffinity labeled avermectin binding site has been purified utilizing a two-step protocol employing gel-exclusion chromatography followed by an immunoaffinity procedure.

C. elegans mRNA injected *Xenopus* oocytes have been used as an expression system to study the interactions between avermectin and the avermectin receptor. Avermectins stimulate a robust inward chloride current in *Xenopus* oocytes injected with *C. elegans* mRNA. Glutamate stimulates the same chloride current and the binding of avermectin to the chloride channel greatly facilitates the interaction of glutamate with its binding site. GABA and GABA antagonists have no effect on this current. *Xenopus* oocytes injected with rat brain mRNA do not respond to avermectin alone, however, avermectin enhances the GABA-induced chloride current. A hypothetical model to explain the mode of action of avermectin will be presented.

Biochemistry of Intermediary Metabolism in Nematodes

I 016 BIOCHEMISTRY AND MOLECULAR BIOLOGY OF GLYCOLYTIC ENZYMES, Ben G. Harris and G.S. Jagannatha Rao, Texas College of Osteopathic Medicine, Fort Worth, TX 76107

Phosphorylation of the *Ascaris suum* phosphofructokinase (PFK) clearly lowers the $S_{0.5}$ value for fructose 6-phosphate (F6P), but this effect alone does not stimulate the enzyme to the extent that it would be active under physiological conditions. PFK has both hysteretic and cooperative properties that can be correlated with each other and with the pKa of a critical histidine residue. These properties can be partially explained by the inhibition of the enzyme by ATP and by pH. However, raising the pH alone does not completely abolish the enzyme's allosteric properties. Derivatization of the *A. suum* PFK with diethylpyrocarbonate in the presence of F6P and MgAMPPCP (a non-metabolizable analog of MgATP) desensitizes the enzyme to the inhibitory properties of ATP. The desensitized enzyme exhibits normal Michaelis-Menten kinetics and is no longer inhibited by ATP. It has been used to define the PFK kinetic mechanism and, more importantly, to establish that phosphorylation of the PFK changes the kinetics of substrate addition, such that F6P is added first. For example, phosphorylation alone changes the K_{iF6P} (where K_{iF6P} represents the dissociation constant of E·F6P) from 50 mM to 150 μM. Since the phosphorylated enzyme is still stimulated by Fru-2,6-P_2 and AMP, there appear to be separate binding sites for these effectors and their effects appear to be synergistic. Therefore, allosteric effectors and phosphorylation can have additional effects on PFK activity, after ATP inhibition of the enzyme is overcome. As described above, phosphorylation, AMP and Fru-2,6-P_2 may all act in concert and affect different aspects of PFK regulation. All three factors must be considered to adequately understand the regulation of PFK activity in parasitic helminths. Recently, PFK has been cloned and sequenced from *H. contortus* by functional complementation in *E. coli* (Klein et al.(1991) Mol. Biochem. Parasitol. 48, 17). The amino acid sequence of the predicted *H. contortus* PFK exhibits about 70% similarity with mammalian PFKs and contains a sequence of 27 conserved amino acids presumed to be part of the active site of the enzyme. Interestingly, no sequence is found at the carboxy-terminus of the *H. contortus* PFK which corresponds to the consensus cAMP-dependent phosphorylation site identified in mammalian PFKs. However, although not noted by the authors, the *H. contortus* PFK does have a sequence near its amino-terminus that contains 7 of the 11 amino acids in the *A. suum* phosphorylated peptide. (Supported by NIH, AI-24155 and the R.A. Welch Foundation, B-0997).

I 017 EVOLUTION OF MITOCHONDRIAL FUMARATE REDUCTASE : COMPARISON OF THE PRIMARY STRUCTURE BETWEEN *ASCARIS* AND *C. ELEGANS*, Kiyoshi Kita[1], Daisuke Mizuchi[1], Hua Wang[1], Toshiaki Kuramochi[1], Shinzaburo Takamiya[2], Takashi Aoki[2], Yuji Kohara[3], and Somei Kojima[1], [1]Tokyo University, Tokyo, Japan, [2]Juntendo University, Tokyo, Japan, [3]National Institute of Genetics, Mishima, Japan.

Complex II (succinate-ubiquinone oxidoreductase) is an important enzyme complex in the tricarboxylic acid cycle and the aerobic respiratory chain of mitochondria and prokaryotic organisms. Complex II catalyzes the oxidation of succinate to fumarate (succinate dehydrogenase: SDH) and transfers its reducing equivalent to ubiquinone in aerobic respiration. Complex II also catalyzes the reduction of fumarate (fumarate reductase: FRD), which is a reverse reaction of SDH activity, in the respiratory chain of anaerobic bacteria and in mitochondria of facultative anaerobic animals such as *Ascaris suum*. Complex II is generally composed of four polypeptides. The largest flavoprotein subunit (Fp) contains covalently bound flavin and the second largest subunit contains iron-sulfur centers (Ip). Two smaller hydrophobic membrane-anchoring peptides (cytochrome *b* subunit; cyb L and cyb S) seem to be essential for converting succinate dehydrogenase to succinate-ubiquinone oxidoreductase. Iron-sulfur centers are the essential prosthetic group for electron transfer in complex II, and three distinct types of iron-sulfur centers are present in complex II: S-1; [2Fe-2S], S-2; [4Fe-4S], and S-3; [3Fe-4S]. Recently, we found a relationship between the high fumarate reductase activity and novel redox properties of the S-3 center in complex II from *Ascaris*, which is a parasitic nematode living in the host's small intestine, where oxygen tension is fairly limited. Clearly, the Ip subunit is a key subunit in complex II, and much more information is required to understand its structure, function and assembly. For this reason, we have started to clone cDNAs for mammalian and nematodes (*Ascaris* and free living *Caenorhabditis elegans*) Ip subunits, and the cDNA clone for human liver Ip has been isolated by immunoscreening a λgt11 cDNA library. In contrast to human liver Ip, no positive clone was found when we tried to screen the *Ascaris* and *C. elegans* cDNA library by immunoscreening. For this reason, we changed our strategy. Mixed oligonucleotide primers corresponding to two conserved regions which appear to be the binding site for the prosthetic group (iron-sulfur centers) were used for cDNA-PCR. Expected size of 480 base pairs of the amplified product were obtained and sequenced directly. Then clones containing full length cDNA were screened by using these PCR products as probes. Striking sequence conservation was found between *Ascaris* and *C. elegans*. From a comparative analysis, the Ip subunit of complex II in *Ascaris* adult appears to be more closely related to those of bacterial SDH than to that of bacterial FRD, even though *Ascaris* complex II shows a high FRD activity.

I 018 ANAEROBIC MITOCHONDRIAL METABOLISM IN PARASITIC HELMINTHS, RICHARD KOMUNIECKI, UNIVERSITY OF TOLEDO, TOLEDO, OHIO 43606

Many parasitic helminths undergo a marked aerobic/anaerobic transition in their energy metabolism during development, and adult tissues have varying capacities to use oxygen as a terminal electron-acceptor. Mitochondria from body wall muscle of the parasitic nematode, *Ascaris suum*, are the most anaerobic of helminth mitochondria and use unsaturated organic acids as terminal electron-acceptors, instead of oxygen. Malate-dependent mitochondrial metabolism involves a reversal of β-oxidation and results in the accumulation of the novel branched-chain fatty acids, 2-methylbutyrate and 2-methylvalerate. At the enzymatic level, a number of key differences which permit significant electron-transport associated energy-generation in the absence of oxygen exist between these anaerobic organelles and aerobic mitochondria. Some enzymes are underexpressed or deleted altogether, such as cytochrome oxidase, citrate synthase and α-ketoglutarate dehydrogenase. Some are overexpressed, such as the pyruvate dehydrogenase complex (PDC), electron-transfer flavoprotein:rhodoquinone oxidoreductase and Complex II. Many have kinetic parameters which are dramatically altered to function under the reducing conditions resulting from decreased oxygen. Surprisingly, even though some of the properties of these enzymes differ dramatically from their aerobic counterparts, they are often quite similar in other respects. For example, the 2-methyl branched-chain enoyl CoA reductase catalyzes a reaction opposite to that of the mammalian acyl CoA dehydrogenases and its substrate specificity, regulation, and mechanism all differ significantly. However, the amino acid sequence of the ascarid enzyme is over 60% identical to the human medium chain acyl CoA dehydrogenase. Most importantly, it appears that many of the key regulatory enzymes exist as aerobic and anaerobic specific isozymes. For example, the regulation and stoichiometry of phosphorylation of the PDC is modified in anaerobic adult muscle and we have identified a specific pyruvate dehydrogenase isozyme that is present in adult muscle but not in earlier aerobic larval stages. These results suggest that the aerobic/anaerobic transition in *A. suum* involves major alterations in the protein composition of its mitochondria.

Enzymes of Other Helminths

I 019 PRENYLATION OF SCHISTOSOME PROTEINS: CHARACTERIZATION OF AN ENZYME WHICH FARNESYLATES LOW MOLECULAR WEIGHT G-PROTEINS, Guo-Zhong Chen, Helen Cirrito and James L. Bennett, Department of Pharmacology, Michigan State University, E. Lansing, MI 48824.

The HMG-CoA reductase inhibitor, lovastatin, can block schistosome egg production and when given chronically to infected mice it has proven to be a potent antischistosomal. The effects of lovastatin can be over come by coadministration of mevalonate, the reaction product of HMG-CoA reductase, but not by the end-products of the mevalonate pathway e.g., cholesterol, dolichols, ubiquinones. The fact that farnesol, another product of the mevalonate pathway, can also overcome the lovastatin induced blockade of *egg production* was unexpected since further elongation of farnesol (a C-15 lipid) is unlikely in the presence of lovastatin e.g., isoprene units necessary for elongation of farnesol require mevalonate. Lovastatin causes swelling of female schistosome cells involved in producing yolk material. Lovastatin treated cells have numerous vesicular organelles suggesting disruption in vesicular trafficking. Vesicular trafficking appears to be regulated, in part, by low molecular weight G-binding proteins (rab proteins). Since prenylation of these proteins appears vital for their function it's our hypothesis that similar proteins function to regulate yolk production in the schistosome.

When schistosomes are incubated in the presence of radiolabeled mevalonate two groups of proteins become labeled, one group around 46 kDa and another around 21-25 kDa. Treatment of these proteins with reagents designed to cleave thioether linkages results in the isolation of two lipids, by HPLC, that have been chromatographically characterized as farnesol and geranylgeraniol.

Farnesyl protein transferase (FPT) and geranylgeranyl protein transferase have been recently identified as the enzymes which can covalently incorporate farnesol (C-15) and geranylgeraniol (C-20) onto cystiene residues located on the carboxy terminus of numerous biologically important macromolecules. We have observed that schistosome homogenates can incorporate radiolabled farnesolpyrophosphate onto 46 kDa schistosome proteins while radiolabled geranylgeranyl-pyrophosphate labels the 21-25 kDa schistosome proteins. FPT activity was dependent upon Mg^{++} and was optimal around pH=8.0. FPT activity was elevated in schistosome homogenates preincubated in mevinolin and the enzyme would actively farnesylate human ras with CVIM or CVLS on the carboxy terminus of the protein but not human ras with SVIM or CVLL on the terminus. The peptide, CVIM, inhibited (IC_{50} ≈ 6 uM) schistosome FPT. A CVIM-farnesol complex was isolated, by HPLC, from the schistosome homogenates incubated in the presence of this peptide. Work on the endogenous schistosome proteins that are farnesylated by FPT will be critical for future studies.

I 020 CHARACTERIZATION OF SMALL NUCLEAR RNAs FROM *SCHISTOSOMA MANSONI*

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We have previously shown that a subset of *S. mansoni* mRNAs contain an identical, 36 nucleotide spliced leader (SL) on their 5' termini, presumably donated to the pre-mRNA from a 90 nucleotide SL-RNA in a trans-splicing reaction. This SL-RNA contains an sm-like binding site which varies from the consensus sequence (AGUUUUCUUUGC versus Pu-A-U_n-G-Pu). In order to determine if this non-consensus sm-binding site in SL-RNA is present in U1-snRNA and to characterize other snRNAs in *S. mansoni*, we developed a method to clone and characterize snRNAs from *S. mansoni* at the cDNA level. Sequencing of multiple *S. mansoni* U1-snRNAs indicated the presence of at least 6 different variants. The sm-binding site in all *S. mansoni* U1-snRNAs is AGUUUUUGU which varies from the consensus sequence in the underlined positions. Immuno-precipitation experiments indicate that U1-snRNAs can be specifically precipitated from *S. mansoni* nuclear extracts with anti-sm serum derived from lupus patients. Conversely, the SL-RNA could not be precipitated using the same anti-sera. With one exception, the altered nucleotide sequences in all of the U1 variants are in regions of the molecule that are generally recognized as non-conserved. One of the variants contains a single nucleotide change (AAACUUACUU versus AAACUUACCU common to the other variants) within the sequence expected to anneal to the 5' splice donor site of pre-mRNA. This altered nucleotide may compensate for a complementary sequence present in multiple 5' splice sites in *S. mansoni* genes, and presumably functions in splicing of these introns. The computer predicted secondary structure of *S. mansoni* U1-snRNA is identical to the conserved secondary structure for U1-snRNA. Also, the sequence of the highly conserved domains in U1-snRNA, including the 5' splice site domain and first loop, are retained in *S. mansoni* U1-snRNA (8-9 out of 10, and 7 out of 8 respectively). The genes encoding U1-snRNA in *S. mansoni* are represented by 800-1200 copies per haploid genome. These genes are mainly arranged in tandem repeats of 1.2 kb, with some copies dispersed throughout the genome. The availability of cloned sequences of major U-snRNAs will enable us to explore interactions between RNA components in nuclear extracts of *S. mansoni*.

I 021 PROTEIN KINASE C (PKC) IN *C. ELEGANS*, Marianne Land, Alma Islas-Trejo, Jonathan H. Freedman, and Charles S. Rubin, Albert Einstein College of Medicine, Bronx, NY 10461.

Certain neurotransmitters, hormones and growth factors activate phospholipase C, which catalyzes the cleavage of phosphatidyl inositol 4,5-bisphosphate to generate diacylglycerol (DAG) and inositol triphosphate (IP_3). DAG and Ca^{2+} mobilized by IP_3 can activate PKC isoforms, which then propagate physiological signals by phosphorylating effector proteins at regulatory sites. Eight mammalian PKC isoforms have been characterized, but little is known about either their exact physiological roles or mechanisms that regulate their expression and intracellular targeting. We are studying the structure, regulation and function of prototypic PKCs in *C. elegans*, a system that provides unique opportunities for elucidating the cellular/molecular basis for PKC diversity and the roles played by PKC isoforms in development and gene regulation. *C. elegans* cDNAs encoding 2 distinct PKC isoforms were cloned and characterized. PKC1 is related to the CNS-specific PKCε isoform of mammals. Heterogeneity is evident at the 5'-ends of PKC1 cDNAs. PKC1 transcripts are either trans-spliced with SL1 (PKC1B) or cis-spliced at the same acceptor site with a 174 nt, 5' extension (PKC1A) that encodes a putative nuclear targeting sequence. PKC1A mRNAs are also trans-spliced at their 5' termini with either SL1, SL2 or novel SLs. Sequences downstream from the cis/trans-splice site are identical. The amino acid sequences of PKC1A (763 residues) and PKC1B (705 residues) contain 2 tandem Zn-finger domains near the N terminus and a 40 kDa C terminal catalytic domain. No Ca^{2+} binding region is evident. Thus, alternative trans/cis splicing serves as a novel mechanism for generating PKC diversity in *C. elegans*. PKC1A and PKC1B mRNAs are differentially expressed during nematode development. PKC1B mRNA is abundant in embryos and L1 larvae, whereas the level of PKC1A mRNA is maximal in adult animals. Affinity-purified IgGs (directed against a shared PKC1 C-terminal region) bind 2 polypeptides (87 and 89 kDa) that presumably correspond to PKC1B and PKC1A, respectively. Both proteins are in the particulate fractions of homogenates from control and phorbol ester-treated *C. elegans*. A segment of the PKC1 gene that includes the cis/trans splice acceptor site and 2.5 kbp of contiguous 5'-flanking DNA was fused to an *E. coli lacZ* reporter gene in a *C. elegans* expression vector. Stable transgenic *C. elegans* carrying this construct expressed β-galactosidase activity only in certain neurons in the nerve ring and tail ganglia. A DNA segment (1.2 kbp) that precedes the cis/trans splice site by >5 kbp, and is also upstream from the sequence encoding the N-terminal nuclear targeting sequence of PKC1A, is being tested as a second promoter for the PKC1 gene. PKC2 is a Ca^{2+}/DAG stimulated enzyme that contains ~ 675 amino acid residues. PKC2 diversity is generated by two mechanisms: Three short alternative sequences appear at the 5' termini of PKC2 cDNAs as the result of the utilization of multiple PKC2 promoters and trans-splicing; In addition, one of two possible 3' terminal exons is excluded from PKC2 mRNA by alternative cis splicing. All internal exon sequences from the PKC2 gene are retained in each PKC2 cDNA. Thus, 6 PKC2 transcripts may be generated from a single gene *in vivo*. Our initial results suggest that it should be possible to elucidate the molecular/cellular basis for PKC diversity in *C. elegans*. Subsequently, specific promoters will be used to target PKC anti-sense constructs, PKC inhibitors, ectopic PKC expression, etc. to selected cells in order to probe the physiological functions of the kinase isoforms.

Molecular Pharmacology of Anthelmintic Drugs

I 022 FUNCTIONAL COMPLEMENTATION OF *E. COLI* STRAINS WITH GENES THAT ENCODE NEMATODE ENZYMES, Timothy G. Geary, M. Anne Favreau, Christal A. Winterrowd, Susan C. Nulf, Susan J. Alexander-Bowman and Ronald D. Klein. Upjohn Laboratories, The Upjohn Company, Kalamazoo, MI 49001.

Parasitic nematodes have evolved pathways of intermediary metabolism that distinguish them from their hosts. Enzymes devoted to energy generation are classic examples. Parasitic nematodes are obligatorily glycolytic, and the rate-limiting enzyme in glucose catabolism, phosphofructokinase (PFK), exhibits distinct biochemical properties in these organisms. A second example is phosphoenolpyruvate carboxykinase (PEPCK), which funnels the 3-carbon products of glycolysis into the nematode mitochondria. In mammals, the primary role of PEPCK is in gluconeogenesis. Kinetic studies of this enzyme are consistent with these contrasting functions. Although it is possible to purify sufficient amounts of nematode PFK and PEPCK for biochemical studies, an understanding of the basis for host-parasite differences requires the determination of amino acid sequence. We have cloned genes that code for PFK from *Haemonchus contortus* and for PEPCK from *H. contortus* and *Ascaris suum*. We employed a strategy of complementation cloning to obtain genes that are functionally expressed. Strains of *E. coli* that are specifically deficient in either PFK or PEPCK and malic enzyme were transformed with parasite cDNA libraries. We selected for transformants that grew in the presence of either mannitol (PFK) or malate (PEPCK) as the sole carbon source. Sequence analysis of plasmids that confirmed the expected phenotype demonstrated that the plasmids contained inserts that encode the targeted genes. Nucleic acid hybridization experiments confirmed that these inserts were of parasite origin. The expressed proteins were characterized enzymatically and by monitoring the flux of [^{13}C]-labeled substrates in complemented bacteria by NMR spectroscopy. The data identify sequence differences that may underlie the kinetic differences that characterize the host and parasite. The parasite enzymes contribute to a carbon flux that is indistinguishable from that of wild-type *E. coli* as determined by NMR spectroscopy.

I 023 UNLICENSED BIOCHEMISTRY AND THE QUEST FOR ANTIHELMINTHICS (SIC), Ronald D. Klein and Timothy G. Geary. Molecular Biology Research and Animal Health Therapeutics, Upjohn Laboratories, Kalamazoo, MI 49001.

Chargaff, nucleic acid chemist and acerbic, accurately defined molecular biology as "the practice of biochemistry without a license," and indeed the inappropriate application of this technology has often confounded rather than solved biological problems. The quest for antihelminthic drugs has been primarily based on whole organism toxicity screens, often employing model organisms, e.g. *C.elegans*. These systems maximize serendipity at significant cost in the pursuit of "false" leads that are either indiscriminant killers, have profound effects on the screening organism but no effect on the target parasite or are simply not pharmacologically relevant. They may also fail to identify leads of significant interest, such as compounds that are not transported into the screening organism for want of minor chemical modification, or that are hidden by potent yet undesirable activities. Scientific advances are beginning to provide information regarding the biochemistry, biology and pharmacology of parasites and host-parasite interactions. The reduction of this ever increasing body of information to drug discovery efforts is a complex and challenging scientific enterprise. Molecular biology has provided tools, through the exploitation of genetically defined organisms, permitting the integration of mechanism-based screening into traditional screening programs. Mechanism-based screens based on mutants complemented with parasite genes can accelerate template discovery efforts by diminishing the number of "false" positives, providing an immediate identity of the target of "true" positives and also providing facile assays of chemically modified drug templates. Facile screening systems and strategies for novel templates specific for nematode glycolytic enzymes and ornithine decarboxylase will be presented. Receptor based screening is another area where drug discovery has been transformed by recombinant DNA technology and mechanistic screening strategies. These screens could be based on ligand-binding assays using parasite tissue where possible, whole parasites or other surrogate tissue sources. These screens are compromised by low receptor abundance and an inability to discriminate between receptor subtypes. Recombinant DNA expression systems using cell cultures and genetically (or "transgenically") defined organisms can provide materials and strategies that specifically address these limitations.

The final question is etymological: how did "antihelminthic" get reduced to "anthelmintic"?

I 024 MOLECULAR PHARMACOLOGY AND DEVELOPMENTAL REGULATION OF HELMINTH PROTEASES, James H. McKerrow[1,2,3,4], Margaret Wasilewski[1], Curtis Loer[1], Celeste Ray[1] and Eugene Sun[1], [1]Departments of Pathology, [2]Medicine, and [3]Pharmaceutical Chemistry, University of California, San Francisco, and [4]Department of Veterans Affairs Medical Center, San Francisco.

Proteolytic enzymes play a variety of roles in the life cycle of free-living and parasitic helminths. We have focused on enzymes of the cysteine protease class, both as targets for drug design as well as probes of gene regulation during helminth development. Cysteine proteases play a key role in digestion of host proteins in the gut of trematodes like *Schistosoma mansoni* and nematodes like *Haemonchus contortus*. Specific fluoromethyl ketone-derivatized peptide inhibitors of the schistosome hemoglobinase were shown to block digestion of host hemoglobin and arrest schistosome development. Utilizing a three-dimensional computer graphics model of the hemoglobinase active site, non-peptide inhibitors were predicted from a database of small molecule structures. The efficacy of the predicted compounds was tested both in assays with the enzyme itself, as well as in an *in vitro* culture system with schistosomula and adult worms. To analyze the function and regulation of this enzyme, a homologue was identified in the free-living nematode *Caenorhabditis elegans*. The *C. elegans* protease was also gut-associated, of the cysteine protease class, and over 50% identical at the primary sequence level to the schistosome enzyme. Regulation of the *C. elegans* protease gene was both temporal (occurring only after larval hatching from the egg) and spatial (only occurring in the gut). Studies to identify promoter elements responsible for temporal and spatial regulation of this gene were initiated by deletion analysis of the upstream region of the protease gene. Various segments of the gut protease promoter were fused to a β-galactosidase reporter gene and transformants scored for location and timing of β-galactosidase expression.

I 025 MOLECULAR PHARMACOLOGY OF NEMATODE BETA-TUBULIN, R. Prichard[1], G. Lubega[1], B. Nare[1], E. Georges[1], R. Beech[1], M. Scott[1], G. Matlashewski[1], R. Klein[2], and T. Geary[2], [1]Institute of Parasitology, McGill University, Ste-Anne-de-Bellevue, Quebec, Canada and [2]Upjohn Laboratories, Kalamazoo, MI, USA.

There is a considerable body of literature on the molecular pharmacology of beta-tubulin of non-nematode species. The molecular pharmacology of nematode beta-tubulin is of particular interest because the benzimidazole (BZ) antiparasitic drugs appear to exert their effects by binding to nematode tubulin and inhibiting polymerization to microtubules. Furthermore, the widespread occurrence of BZ-resistance in nematodes appears to be related to a decrease in the concentration of a high affinity binding (HAB) BZ receptor on *Haemonchus contortus* tubulin. The isoform pattern of beta-tubulins, but not of alpha-tubulins, is altered in resistant strains. The cloning and sequencing of beta-tubulin genes from parasitic nematodes suggests the presence of at least two gene families at different loci. PCR using both general beta-tubulin and loci specific primers for *H. contortus* beta-tubulins, and restriction site analyses of the PCR products are consistent with two loci in this nematode, with multiple alleles at each locus. RFLP and PCR analyses on individual nematodes show a reduced diversity in beta-tubulin alleles, but not alpha-tubulins, in resistant *H. contortus*. Clones of some alleles of each of the two beta-tubulin gene families have been expressed using an *in vitro* expression vector and HAB determined for each gene family. There are distinct differences in the HAB of each gene family. The BZ receptor region of *H. contortus* tubulin has been linked to a BZ analogue by photoaffinity labelling. Sequence analysis of the region of linkage will help us to characterize the HAB receptor region and the alterations associated with loss of the HAB receptor in BZ-resistant nematodes.

I 026 A BIOCHEMICAL APPROACH TO ANTHELMINTIC CHEMOTHERAPY, C. C. Wang[1], Sydney P. Craig III, and Yuan Ling, [1]University of California, San Francisco, CA 94143

Parasitic helminths are, by definition, deficient in metabolic activities, and must rely on the relatively rich environment in the body of a mammalian host for survival and growth. *Schistosoma mansoni* adults are lodged in the portal veins of infected humans. Apparently due to the enriched content of adenosine in human blood, these helminths are able to survive in the bloodstream without the capability of *de novo* synthesis of purine nucleotides. Adenosine is converted by schistosomes primarily to adenine or hypoxanthine which are then incorporated into the nucleotide pool by the actions of adenine and hypoxanthine phosphoribosyltransferase respectively. The latter activity is associated with the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRTase), which recognizes also guanine as its substrate, and converts both purine bases to their corresponding nucleotides. There is little interconversion between adenine and guanine nucleotides in schistosomes, which makes HGPRTase the pivotal enzyme supplying guanine nucleotides to *S. mansoni*. A selective as well as effective inhibition of this enzyme should have an anti-schistosomal therapeutic effect.

S. mansoni HGPRTase was purified to homogeneity by heat treatment and MonoQ column chromatography. It is a dimer with an estimated subunit molecular weight of 26 KDa. Full-length cDNA encoding this enzyme were successfully cloned, sequenced and found to share a 47.9% identity with the cDNA encoding human HGPRTase in a 217 amino acid stretch. The *S. mansoni* cDNA was expressed in *Escherichia coli* using a vector plasmid regulated by a bacterial alkaline phosphatase promoter. The recombinant protein thus generated constitute up to 60% of the total bacterial protein, and stay largely in the soluble, native form. The enzyme is purified to homogeneity with an average yield of 15 to 25 mg per liter of transformed *E. coli* culture. In a similar experiment, the full-length cDNA encoding human HGPRTase was expressed in transformed *E. coli*, and purified in its native form at a similarly high yield. The properties of these two enzyme proteins were compared. The human enzyme is highly heat stable. It retains full activity after 15 min at 85°C, whereas the schistosomal enzyme has a melting temperature of 57.5°C, even though circular dichroism indicated similar α-helical contents (27% and 21%) in the two proteins. A thorough analysis on the steady state kinetics of the schistosomal enzyme-catalyzed reactions concluded on a minimum model of an ordered BiBi mechanism. Thus, where the substrates bind to the enzyme in a defined order (first Mg_2PRPP and then the purine base), and the products are also released in sequence (first MgPPi followed by MgIMP or MgGMP), and vice versa. This ordered release of products is apparently different from that reported previously for human HGPRTase in which the release of products is random. This distinction should allow design of highly specific inhibitors of *S. mansoni* HGPRTase, which would bind exclusively to the enzyme-purine nucleotide binary complex.

We have been collaborating with Dr. Robert Fletterick of UCSF in trying to crystallize *S. mansoni* HGPRTase and human HGPRTase and perform X-ray diffraction analysis of their 3-dimensional structures. High quality crystals of *S. mansoni* (100x300x500 μm) were successfully grown, which generated clearly defined diffraction patterns with a resolution of 2.9A. When the protein was crystallized with 5'-iodoformycin B, the heavy atom derivative yielded clear isomorphous diffraction patterns with a similarly high resolution. The human enzyme was crystallized to a dimension of 1000x150x25 μm which should require further improvement for X-ray diffraction analysis.

When the 3-dimensional structures of the two proteins become available, they will subject to compurter graphic analysis for specific inhibitor design for *S. mansoni* HGPRTase.

Molecular Biology of Helminths

I 027 SPECIFICITY DETERMINATION IN TRANS-SPLICING, Tom Blumenthal, John Spieth, Richard Conrad and Glenn Brooke, Department of Biology, Indiana University, Bloomington.

In *C. elegans* a 22 nt spliced leader (SL) is trans-spliced onto the 5' ends of some, but not all, mRNAs. The SL is donated by a 100 nt RNA, called SL RNA, which exists in the form of a snRNP. *C. elegans* has two SL's, SL1 which is spliced onto the 5' ends of the mRNAs of most trans-spliced genes and is found throughout the nematode phylum, and SL2 which is spliced onto the 5' ends of the mRNAs of a different, smaller set of trans-spliced genes and has been found only in *Caenorhabditis*. Our studies have sought answers to two questions: What marks a pre-mRNA for trans-splicing and where is the information for SL1 vs. SL2 specificity? The answers to the two questions are related in a surprising way: SL1 and SL2 trans-splicing are signalled differently. The signal for SL1 trans-splicing is the presence of an "outron" (intron-like sequence at the 5' end of the pre-mRNA). That is, SL1 trans-spliced genes are different from non-trans-spliced genes in that the former begin with an intron while the latter begin in the conventional way, with the first exon. The signal for SL1 trans-splicing is the presence of a standard splice acceptor site with no splice donor site upstream. We have shown that a conventional gene can be converted into an SL1-accepting trans-spliced gene by inserting an intron from another gene or even synthetic A+U-rich RNA followed by a consensus splice acceptor site into its 5' untranslated region. In addition we have shown that a trans-spliced gene can be converted into a conventional gene simply by insertion of a splice donor site into its outron.

SL2 specific trans-splicing is signalled quite differently. *C. elegans*, unlike all other higher eucaryotes studied to date, has operons (several genes under control of the same promoter). SL2 trans-splicing is specialized for the downstream genes in these operons. It appears that, in most cases at least, no full-length polycistronic mRNA forms: cleavage and polyadenlyation of the upstream gene, accompanied by SL2 trans-splicing of the downstream gene, separate the RNA into single gene units. We don't know the mechanism for SL2 specificity yet, but evidence for the existence of operons takes several forms. We have studied three very closely spaced genes oriented in the same direction. At the 5' end is *mai-1*, which encodes the worm homolog to the mitochondrial ATPase inhibitor, a subunit of the ATP synthase. This gene is separated by only 100 bp from the gene *gpd-2*, which is 100 bp upstream of *gpd-3*, both of which encode GAPDH. There are two promoters, one upstream and another near the 3' end of *mai-1*. *gpd-2* is trans-spliced to both SL1 and SL2 because with one promoter it is the first gene while with the other promoter it is a downstream gene. *gpd-3* is trans-spliced exclusively to SL2 since it is always a downstream gene. Remarkably, polycistronic cDNA clones containing all of *mai-1* and *gpd-2*, with the introns spliced out and polyadenylated at the end of *gpd-2* have been isolated. These may represent processing intermediates on the way to mature mRNAs, based on the finding of *mai-1* cDNA clones, polyadenylated at the trans-splice site of *gpd-2*. Other evidence for the existence of the operon includes a demonstration that a heat shock promoter placed upstream of *gpd-2* results in strictly heat-inducible expression of SL2-trans-spliced *gpd-3*, while placements of the heat shock promoter closer to the 5' end of *gpd-3* result in loss of SL2 specificity. Deletion of the AAUAAA polyadenylation signal results in accumulation of a polycistronic RNA. Furthermore, the specificity of an SL1-accepting gene can be changed to SL2 by placing it into the position normally occupied by *gpd-3*, even though it is preceded by its own outron sequence. Finally, four other SL2-accepting genes have been shown to be preceded by genes just upstream oriented in the same direction. We believe that events that occur at the cleavage and polyadenylation site of the upstream gene are somehow involved in trans-splicing of SL2 to the downstream gene. These results have important implications for mechanisms of splicing, polyadenylation and gene regulation in nematodes.

I 028 STRUCTURAL AND FUNCTIONAL CONGRUITIES BETWEEN THE NEMATODE *CAENORHABDITIS ELEGANS* AND PARASITIC NEMATODES, Carl D. Johnson, NemaPharm, Inc., Cambridge, MA 02139

As a result of recent efforts, the free-living soil nematode *Caenorhabditis elegans* is probably the most thoroughly understood animal in terms of its anatomy, development and behavior. In addition, the genes of *C. elegans* are accessible through powerful, well-developed and well-practiced classical and molecular genetic technologies, and mapping and sequencing of the *C. elegans* is advancing rapidly. A striking conclusion from the accumulated knowledge about *C. elegans* is that many aspects of anatomy, physiology and development observed in this nematode are closely related to those of economically and medically significant parasitic nematodes. Investigations of *C. elegans* can be expected to assist endeavors designed to control parasitic nematodes by:

i) contributing to the study of the mode-of-action, including identification of targets, for existing anti-nematode drugs,
ii) defining and characterizing targets for new drugs,
iii) facilitating the discovery and development of new drugs and
iv) exploring the properties and mechanisms of resistance to drugs.

Results from two areas of research -- biochemical studies of nematode acetylcholinesterases and comparative neuroanatomy of nematodes -- illustrate detailed homologies between *C. elegans* and parasitic nematodes. More recent research has analyzed in detail the frequency and properties of ivermectin resistance in *C. elegans*. In brief, the results show that low-level (4-10X) ivermectin resistance is very common and can result from mutation in >25 *a*vermectin *r*esistance (*avr*) genes. In contrast higher-level (>50X) resistance is very rare, requiring separate mutations in two different *avr* genes. From these results a hypothesis regarding the potential for and properties of ivermectin resistance in parasitic nematodes has been developed. Current experiments are testing the hypothesis by isolation and analysis of ivermectin resistant strains from four additional species of free-living nematodes. The goals of this research are twofold. First, the knowledge gained will assist in the formulation of ivermectin use policies that place appropriate emphasis on procedures designed to minimize the selection of ivermectin resistant parasites. Second, the identification of *avr* genes that appear most likely to be responsible for ivermectin resistance in parasitic nematodes, will facilitate development of diagnostic reagents for detecting nascent resistance and provide a screen for new treatments capable of arresting, and preferable reversing, the growth of the ivermectin resistant fraction of the population. (Supported by grants AI32280 and RR07734 from the NIH and TSA #910625 from WHO.)

I 029 GENETICS OF PARASITIC NEMATODES, Leo F. Le Jambre, CSIRO Division of Animal Health, Pastoral Research Laboratory, Armidale, NSW 2350, Australia.

The benefits to parasite control that would accrue from being able to identify anthelmintic resistance genes before they become widespread has provided a great impetus to the study of the genetics of parasitic nematodes. The anthelmintic properties of the benzimidazoles (BZs) are due to their binding to ß-tubulin and preventing microtubule formation. The sequence of parasitic nematode ß-tubulin genes has been determined and the information used to develop a polymerase chain reaction (PCR) assay capable of detecting resistance in a single larvae. Two ß-tubulin isotypes appear to widespread in the Family Trichostongylidae including *Ostertagia circumcincta, Trichostrongylus vitrinus, Haemonchus contortus* and *Trichostrongylus colubriformis*. Unlike the BZs, the resistance mechanism to ivermectin is not known; and hence, the genes encoding for it also are unknown. An important first step towards identifying the resistance genes was first to identify the mode of inheritance in crosses made between resistant and susceptible parasites. Resistance was found to be autosomal dominant in *H. contortus* and polygenic in *T. colubriformis* (Martin and Turney, pers. comm.). Two approaches are being followed in the study of the molecular genetics of ivemectin resistance in parasitic nematodes. The first aims to isolate the resistance gene in an inbred background through a series of backcrosses to an inbred strain of *H. contortus*. DNA polymorphisms would then be linked to resistance. The second aims to determine the linkage group of the resistant gene with sequence tagged microsatellite sites (STMSs). *H. contortus* and *T. colubriformis* genomic libraries were constructed in a phagemid vector for convenient isolation and of STMS. Screening of these libraries followed by sequencing and PCR analysis confirmed the presence of GT, GA or ATT repeat microsatellites in both species. To determine function of resistance genes, our laboratory (1) is developing a transformation system based on *Caenorhabditis elegans*. *C. elegans* has been transformed with the ß-tubulin gene from *T. colubriformis* implicated in BZ resistance by RFLP analysis. The gene is being expressed in the transformants and they display an increased susceptibility to BZs.

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I 030 TWO DIFFERENT CU/ZN SUPEROXIDE DISMUTASES IN SCHISTOSOMA MANSONI. Zhi Hong[1], Arvind Thakur[2], Hai-Ping Mei[2], David Kosman[1], Marie-Louise Hammarskjold[2], David Rekosh[1,2], and Philip LoVerde[2]. Department of [1]Biochemistry and [2]Microbiology, State University of New York, Buffalo, NY 14214.

We have previously characterized a gene in S. mansoni which putatively encodes a Cu/Zn superoxide dismutase. Its predicted gene product contains a hydrophobic leader peptide, as well as an N-linked glycosylation site, which suggests an extracellular or membrane-associated form. Thus we have designated it signal peptide-containing (SP) SOD. We expressed the gene in COS cells and E. coli, but failed to detect SOD activity. However, in tunicamycin studies, we showed on western blot, using specific monoclonal antibodies, that the putative SP-SOD expressed in COS cells is N-linked glycosylated. To demonstrate that our cloned gene product is SOD, we directly assayed SOD activity in the parasite and used specific monoclonal antibodies to confirm that the activity correlated with the presence of our gene product. Although our results showed that most of the SOD activity was in a surface associated fraction of the adult worm, surprisingly, this fraction was not immunoreactive. Tissue fractionation experiments also indicated that most of the immunoreactive gene product was not extractable from the parasite with detergent. These apparent contradictions lead us to the identification of another Cu/Zn SOD from S. mansoni. A 16kD protein was purified to homogeneity and three peptides derived from it were sequenced. All the peptides share homology with the 19 other sequenced Cu/Zn SODs. Degenerate oligonucleotides designed according to the sequence and position of each peptide were used to isolate the gene by PCR using Schistosoma RNA as the template. The protein encoded by the cDNA has 153 amino acids with a calculated molecular weight of 15,963. It has 60-65% homology to 19 cytosolic Cu/Zn SODs from various species. Amino acid sequence comparison between the two schistosome SOD sequences shows a 45% homology and demonstrates there are two Cu/Zn SODs, each encoded by a different gene. Overexpression of the 16kD Cu/Zn SOD in bacteria resulted in a gene product that showed enzyme activity comparable to the native enzyme. (Supported by AI18867)

I 031 *TRANS*-SPLICING OF NEMATODE PRE-mRNA, Timothy W. Nilsen, Patricia A. Maroney, Gregory J. Hannon, Yi-Tao Yu; Case Western Reserve University School of Medicine, Cleveland, Ohio USA 44106-4960.

Biochemical analysis of nematode *trans*-splicing has been facilitated by the availability of a homologous *in vitro trans*-splicing system prepared from developing embryos of the parasitic nematode *Ascaris*. Since synthetic SL RNA can serve as the *trans*-splice donor in this cell-free system, it has been possible to use mutational analysis to identify sequences within the SL RNA that are important for its function. Such analysis has shown that neither exon sequence nor secondary structure is important for SL RNA function *in vitro*. Thus, determinants of SL RNA function (in addition to a 5' splice site) reside within the snRNA-like domain of the molecule. Chemical modification interference analysis was used to define critical purine residues within the snRNA-like domain. This experiment identified nine individual purines that were essential for SL RNA activity. These residues were clustered in two regions of the molecule, a short stretch on the 3' side of stem II and the single stranded region between stems II and III. This single stranded region contains the SL RNA's Sm binding site and adjacent nucleotides. The importance of the sequence elements identified by modification interference was confirmed by their ability in combination to confer SL RNA function to a fragment of an Ascaris U1 snRNA.

The functional significance of the single-stranded region containing the Sm binding site and adjacent nucleotides has been examined further. Somewhat surprisingly, chemical modification of any one of the Sm binding site purines did not inhibit assembly of the SL RNA into an Sm RNP. This observation, coupled with the observation that a functional Sm binding site derived from U1 snRNA (AAUUUUUGC) could not substitute for the analagous sequence in the SL RNA (AAUUUUGG), suggested that the Sm binding region of the SL RNA had a role in addition to directing assembly into an Sm RNP. To address the possibility that the significance of these sequences might (at least in part) reside in their ability to interact with another RNA, crosslinking experiments using aminomethyltrioxsalen (AMT) were performed.

Cross-linked species of identical mobility were observed with labelled synthetic SL RNA (as assayed by gel electrophoresis) or endogenous SL RNA (as assayed by Northern blot). Synthetic SL RNAs altered in the critical nucleotides 3' of the Sm binding site efficiently assemble into Sm RNPs but fail to function in *trans*-splicing. These same mutant SL RNAs fail to crosslink, suggesting that the crosslink is functionally significant. To determine if the crosslink resulted from an interaction between the SL RNA and a known snRNA, crosslinked species were digested with RNase H using a panel of oligodeoxynucleotides complementary to U1, U2, U4, U5 and U6 snRNAs. The crosslinked moieties were sensitive to RNase H in the presence of two separate oligodeoxynucletodies complementary to U6 snRNA and insensitive to digestion with any of the other nucleotides. Inspection of the SL RNA and U6 snRNA sequences revealed a striking complementarity of 18 consecutive base pairs with one bulged nucleotide. In the SL RNA, the region of the complementarity includes the Sm binding site and extends 10 bases 3' of the Sm binding site. In U6 snRNA the region of complementarity comprises bases 83-100 and thus includes the region of U6 which has been shown in mammalian cells to interact with U2 snRNA. Mapping by RNA fingerprinting and partial alkaline hydrolysis placed an AMT crosslink within this region of complementarity (U98 of U6 snRNA and U77 of the SL RNA). U77 is the 5' most uridine residue of the SL RNA's Sm binding site.

These results suggest that a SL RNA/U6 snRNA base pairing interaction may be important for *trans*-splicing and further suggest a possible mechanism whereby the two substrates for *trans*-splicing (the SL RNA and acceptor pre-mRNA) can efficiently associate in the absence of any sequence complementarity to each other. It is well established that U2 snRNA interacts by base-pairing with the branch-site of pre-mRNAs. Recently, the functional significance of a U2/U6 base pairing interaction has been demonstrated in mammalian *cis*-splicing. These interactions together with the SL RNA/U6 snRNA interaction described above, provide a plausible model of *trans*-spliceosome assembly where U6 snRNA and U2 snRNA provide a connecting bridge between the SL RNA and acceptor pre-mRNA.

Functional Molecular Biology of C. Elegans

I 032 SITE SELECTED INSERTION OF THE *C. elegans* TRANSPOSABLE ELEMENT Tc1 INTO SPECIFIC MUSCLE GENES. Alice Rushforth, Claudia Cummins, Bonnie Saari, and Phil Anderson. Department of Genetics, University of Wisconsin, Madison, WI, 53706.

We have developed a general method for identifying transposon-induced alleles of *C. elegans* genes that have been previously sequenced but whose mutant phenotypes are unknown. The method uses the Polymerase Chain Reaction (PCR) to identify nematode populations containing rare Tc1 insertions into a chosen gene. Tc1 is one of several families of active transposable elements in *C. elegans*, and in certain strains, most spontaneous mutations are caused by insertion of Tc1. After identifying populations of animals that contain an insertion of interest, the desired mutants are isolated from these populations using a sib-selection protocol.

We used this method to isolate Tc1 insertion alleles of *mlc-2*, one of two *C. elegans* regulatory myosin light chain genes, and *hlh-1*, a *C. elegans* homolog of the vertebrate MyoD myogenic regulatory factor. Our goals were (i.) to develop a general method for identifying mutations in any sequenced gene and (ii.) to establish the phenotype of *mlc-2* and *hlh-1* loss-of-function mutants. We identified nematode populations that contained either *mlc-2::Tc1* or *hlh-1::Tc1* mutants, and, after several cycles of subdivision and retesting, isolated the desired *mlc-2::Tc1* or *hlh-1::Tc1* homozygotes. We obtained three independent insertions of Tc1 into *mlc-2* and two independent insertions into *hlh-1*. All of these mutants are essentially wild-type when homozygous.

The wild-type phenotype of these insertion mutants would ordinarily indicate that function of *mlc-2* or *hlh-1* is unnecessary or redundant. This conclusion is valid, however, only if the insertion mutations are null alleles of the gene involved. In fact, it appears that all of our *mlc-2::Tc1* and *hlh-1::Tc1* mutants are NOT null alleles, even though they contain insertion of Tc1 (1.6 kb.) within exons of the target gene. Each of the *mlc-2::Tc1* mutants contains small amounts of *mlc-2* mRNA in which all or part of Tc1 is spliced from the pre-mRNA, leaving small in-frame insertions or deletions in the mature message. Thus, the *mlc-2::Tc1* mutants express small amounts of a qualitatively altered MLC protein. Similar RNA processing removes all or part of Tc1 from the *hlh-2::Tc1* pre-mRNA. There is a remarkable plasticity in the splice sites that are used to remove Tc1 from these pre-mRNAs. Certain 5' and 3' splice sites that are activated and utilized in the mutants are very unlike typical eukaryotic splice sites.

I 033 MUSCLE DEVELOPMENT IN *C. ELEGANS*, Peter G. Okkema, Verena Plunger, Lihsia Chen, Joohong Ahnn, and Andrew Fire, Carnegie Institution of Washington, Department of Embryology, Baltimore, MD 21210.

The four myosin heavy chain genes of *C. elegans* are differentially expressed in two major muscle types: *myo-1* and *myo-2* are expressed in pharyngeal muscle; *myo-3* and *unc-54* are expressed in body wall muscle. We are characterizing *cis*-acting regulatory sites and *trans*-acting factors controlling *myo-2* and *unc-54* expression to identify determinants of pharyngeal and body wall muscle fate.

We analyzed *myo-2* and *unc-54* regulatory sequences using *lacZ* gene fusions. Each gene contains separable tissue-specific enhancer and promoter elements. The enhancers in turn contain multiple elements which cooperatively activate transcription. Two short segments in the *myo-2* enhancer activate transcription in overlapping sets of pharyngeal cells: "B" can activate in a subset of pharyngeal muscles; while "C" can activate in all pharyngeal muscles and some non-muscle pharyngeal cells. We therefore propose tissue-specificity of the *myo-2* enhancer results from a pharyngeal muscle-specific element and an organ-specific element. Four sites in the *unc-54* enhancer (designated O, I, II, and III) are necessary for activity. Novel combinations of these four sites can activate in muscle and non-muscle, suggesting body wall muscle-specificity of the *unc-54* enhancer is generated by a combination of activators with broader tissue distributions.

A cDNA expression library was screened with *myo-2* and *unc-54* enhancer elements. Two genes encoding products specifically binding the *myo-2* B element were isolated. One contains a homeodomain highly related (83% identical) to the NK-2 gene of *Drosophila*. The second has no conserved DNA binding motif but shares some features with b-ZIP and b-hlh proteins. The two factors apparently bind the left and right half of the B element, respectively, and both proteins may be necessary for activity. Two genes encoding products specifically binding the *unc-54* enhancer were also isolated. One encodes a member of the Y-box family of transcription factors; and the second encodes a zinc-finger protein. The Y-box factor binds sites O and I; while the zinc-finger protein binds site III. We are currently raising antibodies and constructing *lacZ* fusions to determine the tissue distribution of these factors.

Two additional approaches to identify factors controlling muscle development are being pursued. First, *C. elegans* homologs of vertebrate myogenic factors are being isolated and functionally characterized. Second, a genetic screen is being undertaken to identify zygotic requirements for muscle differentiation.

I 034 RECEPTORS CONTROLLING C. ELEGANS DAUER LARVA DEVELOPMENT, Donald L. Riddle, Miguel Estevez, Wen-Hui Yeh, and Patrice S. Albert, Molecular Biology Program, University of Missouri, Columbia, MO 65211.

Starvation and overcrowding of *C. elegans* during the first larval stage induces formation of developmentally arrested, non-feeding dauer larvae at the second molt. Dauer larvae may survive for months, and when conditions favorable for growth are found, they resume development to adulthood. Infective stages of some parasitic nematodes are closely analogous to the *C.elegans* dauer larva. The developmental decisions governing entry into, or exit from, the dauer stage are made in response to the ratio of food to the *C. elegans* dauer-inducing pheromone in the environment (1). Mutants affected in the decision to form dauer larvae are either dauer-constitutive, which form dauer larvae in abundant food, or dauer-defective, which cannot form dauer larvae when starved. Interactions between specific dauer-constitutive and dauer-defective mutants have been used to construct pathways for gene action, now including more than 20 genes. One branch of our pathway is temperature-dependent, another is not. Four genes have been cloned by transposon-tagging, and three of these encode receptor molecules. Intermediate steps in the genetic pathway are mediated by the *daf-1* and *daf-4* genes. Both of these genes encode transmembrane receptor serine kinases, which are expressed at all stages. Both receptors are required for non-dauer development and for normal egg laying in the adult. The *daf-1* receptor was the first receptor serine kinase reported, and mammalian activin and TGF-β receptors subsequently have been found to share a number of sequence motifs in common with it. The *daf-4* gene encodes a kinase 33% identical to the kinase domain of the *daf-1* receptor kinase (2) and 40% identical to the kinase domain of the mouse activin receptor (3). Activin and TGF-β are morphogens important in vertebrate development. If the signal transduction pathway that regulates development of *C. elegans* dauer larvae is the nematode analogue of a TGF-β or activin signalling system, the genetic analysis in *C. elegans* may provide the basis for identifying the missing links between signal generation and control of gene expression in vertebrate systems.

The *daf-12* gene specifies what we believe to be the last step in signal transduction. It encodes a member of the steroid/thyroid hormone receptor superfamily. Its sequence appears to be most similar to the human vitamin D and retinoic acid receptors. Whereas *daf-1* and *daf-4* are required for normal non-dauer development, *daf-12* activity is required for dauer larva morphogenesis. We propose that the activin-receptor-like *daf* kinases phosphorylate proteins that promote growth, and directly or indirectly inactivate the *daf-12* receptor, possibly by preventing synthesis of a dauer-inducing hormone. Receptors similar to those encoded by *daf-1*, *daf-4*, and *daf-12* are presumably also present in other nematode species, and they are potential points of intervention in parasite life cycles.

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3. Matthews, L., and W. Vale. 1991. Cell 65:973-982.

I 035 DNA SEQUENCE ANALYSIS OF THE C. ELEGANS GENOME. R. K. Wilson (1), K. Anderson (1), R. Ainscough (2), L. Antonacci-Fulton (1), M. Berks (2), J. Cooper (1), M. Connell (1), A. Coulson (2), M. Craxton (2), S. Dear (2), Z. Du (1), R. Durbin (2), A.Favello (1), P. Green (1), J. Hawkins (1), T. Hawkins (2), L. Hillier (1), C. Huynh (1), L. Johnston (1), J. Kershaw (2), J. Kirsten (1), Y. Kozono (1), N. Laister (2), J. Latreille (1), C. Martin (4), S. Ramachandra (1), A. Roopra (1), R. Shownkeen (2), N. Smaldon (2), A. Smith (2), R. Staden (2), J. Sulston (2), J. Thierry-Mieg (3), K. Thomas (2), M. Vaudin (1), R. Waterston (1), P. Wohldmann (1) and J. Zhu (1). (1) Department of Genetics, Washington University School of Medicine,St. Louis, MO 63110, USA. (2) M.R.C. Laboratory of Molecular Biology, Cambridge CB2 2QH, England. (3) CNRS-CRBM et Physique-Mathematique,Montpellier 34044, France. (4) Human Genome Center, Building 74, Lawrence Berkeley Laboratory, Berkeley, California 94720, USA.

The haploid genome of C. elegans contains about 100 Mb, distributed over six chromosomes. Most of the genome is covered by a map consisting of 17,500 cosmids and 3,500 YAC clones. Over 90 Mb is contained in 22 large contigs assigned to chromosomal locations; most of the rest are represented by 23 smaller contigs. The remaining gaps are probably small. This physical map is now being used for DNA sequence analysis of the C. elegans genome, commencing with a three year pilot project to sequence a contiguous region of 3 Mb on chromosome III. The approach being used is "shotgun" analysis of 1-2 kb inserts in M13 and 6-9kb inserts in phagemids, followed by a walking phase to completion. Data collected by ABI 373A fluorescent gel readers is assembled using the program XDAP, which has greatly facilitated the editing process by allowing instant viewing of primary sequence data. After two years of the project, results include the complete sequence of over 1 Mb, with additional regions at various stages of assembly. Analysis of the completed regions by GENEFINDER has revealed an average gene density of approximately one per 5 kb. Comparison with the public sequence databases by BLAST reveals similarities for one gene in three; recent additions to the list include: cyclin, ras-like protein, three zinc-finger proteins, integrin, glutamate and secretin receptors, heat shock protein, NFkB-transcription factor, GTPase activating protein, and K+ channel. Continued development of our assembly software has now led to a fully automated package (SQUIRREL), which grades primary data, clips the raw sequences and performs shotgun assembly without operator intervention. At present, all editing is done interactively, but improved basecalling algorithms should allow much of it to be automated in the near future. The operator will then only have to deal with failed clones and devise strategies for contiguation and double-stranding. In addition to software development, automation is being developed for template preparation and sequencing reactions. The primary repository for the C. elegans data is the database management program ACEDB. All data is then released to the EMBL or GenBank databases. As an adjunct to the genomic sequencing project, a sorted library of 1517 cDNAs has been tag-sequenced to obtain a 5' sequence read from each clone. Through hybridization to YAC grids, the positions of over 98% of the cDNA clones have been located on the genome map.

Late Abstract

C. ELEGANS NEURONAL DIFFERENTIATION AND DEGENERATION, Martin Chalfie, Columbia University, New York.

The *mec-3* gene of *C. elegans* is needed for the proper differentiation of a set of six touch receptor neurons. In *mec-3* mutants the cells that would normally develop as these receptors still differentiate as neurons, but fail to express any of the touch-cell-specific features. An important aspect of *mec-3* function is that the protein, a LIM-type homeodomain protein, forms heretodimers with a second homeodomain protein, the product of the *unc-86* gene. Because *mec-3* and *unc-86* are expressed in the touch cells and two other pairs of neurons, other gene products must act with these to specify the touch-cell fate. By examining mutant animals for the appearance of ectopic cells expressing touch cell features, we have indentified several genes whose products act in combination with *mec-3* (and *unc-86*) to specify which cells become touch cells. The simplest model is one in which *mec-3*, *unc-86*, and *lin-14* all act positively to promote touch cell differentiation. Other cells are prevented from developing this fate by negative regulation of *lin-14* (by *lin-4*), *mec-3* (by *sem-4*), ordownstream genes (*egl-44*, *egl-46*). A further restriction in the number of touch cells results from programmed cell death. Analysis of touch cell mutants has also allowed us to examine inherited neurodegeneration. Dominant mutations of the *mec-4* gene lead to the death, by a process that is morphologically and genetically distinct from programmed cell death, of the touch cells. *mec-4* helps define a family of genes (the degenerin family) of putative membrane proteins. A new member of this family is also a touch cell gene, the gene *mec-10*. Interestingly, a mutation that results in the *mec-4*-induced deaths, does not cause degeneration when placed in *mec-10*.

Surface Biology and Immunology

I 100 EPICUTICULAR LIPID COMPOSITON OF WILD-TYPE AND MUTANT *CAENORHABDITIS ELEGANS*
Mark L. Blaxter, Wellcome Centre for Parasitic Infections, Department of Biology, Imperial College of Science, Technology and Medicine, London SW7 2BB, UK.

Ultrastructurally, the nematode cuticle is bounded by a lower membrane (the plasma membrane of the hypodermal syncytium) and an apical, electron-dense lipid structure, the epicuticle. The epicuticle displays some properties associated with lipid bilayers, but also has features suggestive of radically different organisation. The peculiar properties of the epicuticle may be imparted by its lipid makeup, and understanding of the components of the epicuticle may aid in understanding its biological function, and relation to the non-collagenous cuticle proteins identified in both parasitic nematodes and the free-living *Caenorhabditis elegans*. Surface radioiodination using iodogen was used to label the unsaturated lipids of the surface of adult and larval *C. elegans*, and high performance TLC used to analyse the labeled lipids. 20 different species of lipid, including cholesterols, phospholipids and complex lipids, were identified. Comparison of total with surface lipid composition revealed that the major *C. elegans* glycolipids are not surface exposed. L1, L3 and adult nematodes differed in their surface lipid profiles. The *srf* mutants of *C. elgans* have novel surface lectin-binding properties compared to wild type, and some also have developmental and locomotor defects. Specific changes in the surface lipid profile of both nonpleiotropic (*srf-2*, *srf-3* and *srf-5*) and pleiotropic (*srf-4*, *srf-8* and *srf-9*) mutants were seen.

I 101 EXCRETORY-SECRETORY SUPEROXIDE DISMUTASE (SOD) OF THE BOVINE LUNGWORM *DICTYOCAULUS VIVIPARUS*.
Collette Britton, David P. Knox* and Malcolm W. Kennedy. Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Glasgow, Scotland, U.K. and *Moredun Research Institute, Edinburgh, Scotland, U.K.

A number of parasitic helminths are known to secrete one or more anti-oxidant enzymes into their immediate environment. Such enzymes are thought to play an important role in protecting the parasites against toxic oxidants generated by host phagocytes in response to infection. As yet, there is no information on anti-oxidant enzymes of the bovine lungworm *D. viviparus*, the adult stage of which resides in the high oxygen environment of the air spaces of the lungs and induces a significant mast cell response. High levels of superoxide dismutase (SOD) were detected in *in vitro*-released products of adult *D. viviparus* in assays using the xanthine-xanthine oxidase system and by non-denaturing PAGE followed by incubation in nitro blue tetrazolium in the presence of superoxide. Three isozymes were identified, all of which were sensitive to cyanide, indicating copper/zinc dependency. Moreover, purified IgG from bovine hosts exposed to infection with normal larvae or vaccination with X-irradiated larvae inhibited *D. viviparus* SOD activity by 70%, indicating that SOD is a target of the host immune reponse to *D. viviparus*. Inhibition of SOD activity *in vivo* may, therefore, be involved in limiting parasite survival within the host.

I 102 IDENTIFICATION OF A NOVEL REPETITIVE ANTIGEN COMMON TO *ONCHOCERCA GIBSONI* AND *O.VOLVULUS*,
Julian Catmull, Dan Zhang, Florence Ruggiero and David J. Miller. Department of Chemistry and Biochemistry, James Cook University of North Queensland, Townsville., 4811. QLD., Australia.

We have constructed stage-specific cDNA libraries from mRNA isolated from uterine microfilariae and from eggs of *O. gibsoni*. In general, this cattle parasite is the favoured animal model for the human pathogen *O.volvulus*. We are in the process of characterising the mRNA complement of microfilariae and eggs of *Onchocerca gibsoni*, From these cDNA libraries a clone was isolated encoding an antigen which was recognised strongly by serum from individuals infected with *O.volvulus* and which was found to contain five copies of an 87 bp repeat unit. Recombinant protein corresponding to this cDNA was synthesised using the pGEX system. Antibodies raised to this recombinant protein were used to screen Donelson's *O.volvulus* cDNA libraries and also for immunolocalisation and western blotting experiments. The *O.volvulus* clones were found to contain repeating regions of high similarity to those present in *O.gibsoni*, interspersed with other repetitive regions which had no equivalents in *O.gibsoni*. Immunolocalisation and western blotting experiments imply that these cDNAs encode a high molecular weight membrane-associated antigen. In ELISA experiments, serum from individuals infected with *Wuchereria bancrofti*, *Loa loa*, and *Brugia malayi* also reacted strongly with the recombinant *O.gibsoni* protein, implying that the antigen may be common to a wide range of filarial parasites.

I 103 IDENTIFICATION OF A cDNA CLONE THAT CODES FOR A CIRCULATING *ONCHOCERCA VOLVULUS* ANTIGEN,
Ramaswamy Chandrashekar, Kurt Curtis and Gary J. Weil, Departments of Medicine and Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110

We have previously identified parasite antigens that are present in immune complexes in sera from some onchocerciasis patients, and we have produced monoclonal antibodies (OV-1 and OV-5) that bind to these antigens. The purpose of this study was to clone circulating *O. volvulus* antigens. An *O. volvulus* adult worm lambda gt11 cDNA library was immunoscreened with OV-1 and OV-5. The most immunoreactive of 5 clones (OV1C) was chosen for further study. Antibodies affinity purified from human onchocerciasis sera with OV1C fusion protein bound to the same 55 kDa *O. volvulus* adult antigen in Western blots as monoclonal OV-1. Sequence analysis showed that OV1C is a partial length cDNA with an ORF that codes for 98 amino acids. The protein encoded by OV1C is highly hydrophilic, and it has no predicted glycosylation sites. No homology with other clones was noted in a GenBank search. The library was reprobed with HRP-labeled OV1C DNA in an attempt to isolate a full-length cDNA clone. Two clones that hybridized with the OV1C DNA probe were purified by immunoscreening with OV-1 antibody. Both clones have 1.8 kb cDNA inserts. One clone has been partially sequenced, and it contains the OV1C sequence. Future studies will attempt to understand the function of this *O. volvulus* circulating antigen and explore whether this putative recombinant antigen is useful for diagnosis of onchocerciasis.

I 104 PURIFICATION OF COOPERIA ONCOPHORA SPECIFIC ANTIGENS FROM ADULT WORM EXTRACTS AND ITS APPLICATION IN AN ENZYME LINKED IMMUNOSORBENT ASSAY.

D.C. de Graaf, H. Hilderson, P. Berghen and J. Vercruysse, Laboratory of Parasitology, Faculty of Veterinary Medicine, University of Gent, Casinoplein 24, 9000 Gent, Belgium

Cooperia oncophora and *Ostertagia ostertagi* are both economical important gastrointestinal parasites of cattle in temperate regions. The current diagnostic enzyme linked immunoassay for these nematodes is based on total worm extracts. In this communication the attempts are presented to improve the specificity of this assay. Total adult worm extracts were screened for species specific antigens by Western blot analysis with sera from *C. oncophora* and *O. ostertagi* monoinfected calves. Two antigens with MW 14.2 and 14.9 kDa were only recognized by serum of *C. oncophora* monoinfected calves. The purification of these species specific antigens was achieved by gelfiltration and ionexchange chromatography. The purified antigens were applied for the development an enzyme linked immunoso.bent assay. The evaluation of this assay was done with pooled serum of parasite naive animals and *Cooperia* and *Ostertagia* monoinfected calves. It was demonstrated that the application of purified *Cooperia* antigens resulted in an increased specificity of this assay, when compared to total worm extracts.

I 105 CHARACTERIZATION OF TWO LARVAL EXCRETORY-SECRETORY PROTEINS OF DIROFILARIA IMMITIS RELEASED AT THE TIME OF THE THIRD MOLT, Glenn R. Frank and Robert B. Grieve, Department of Pathology, Colorado State University and Paravax, Inc., Fort Collins, CO 80523

Two proteins have been described in the excretory-secretory products (ES) collected from D. immitis during the molt from the third stage to the fourth stage in vitro. During the purification of these two proteins a third protein has been identified. All have molecular weights between 20 and 23 kDa as determined by Tris-glycine SDS-PAGE and have been designated as 20, 22L and 22U kDa proteins. The three proteins were purified from larval ES collected under serum free conditions using cation exchange followed by C4 reverse phase HPLC. All were subjected to trypsin digestion and their peptides separated by C18 reverse phase HPLC. Amino acid sequences were determined on at least three peptides from each protein and the N-terminus of the 20 kDa protein. The 20 and 22L kDA proteins appear to be quite similar based on the difficulty in separation during purification and sequence similarity. The 22U kDa protein has also been identified in adults using tryptic mapping after the same purification scheme, and amino acid sequencing of one peptide. The 20 and 22L kDa proteins were recognized by sera from dogs immune to infection by D. immitis but not sera from infected non-immune dogs as determined by immunoblot analysis. Oligomeric DNA probes have been synthesized for northern blot analysis and the cloning of these molecules from larval cDNA libraries.

I 106 CLONING AND CHARACTERIZATION OF A MAJOR SURFACE GLYCOPROTEIN (Gp29) IN *DIROFILARIA IMMITIS*, Rexann S. Frank, Cynthia A. Tripp, Murray E. Selkirk, Marcia M. Grieve, and Robert B. Grieve, Department of Pathology, Colorado State University, Fort Collins, CO 80523

One proposed mechanism of immune evasion by filarial parasites is the inactivation of the mammalian leukocyte oxidative burst by glutathione peroxidases. Such a defense mechanism is an attractive target for immunoprophylaxis. A highly conserved 29 kD glutathione peroxidase (Gp29) is a major surface glycoprotein found in the adult and larval stages of several filarial parasites, including *D.immitis*. Immunoblot analysis with a polyclonal anti-Gp29 serum detected a 29 kD protein in soluble adult, third stage (L3) and fourth stage (L4) antigens and 29 and 55 kD bands in larval excretory/ secretory products. The gene encoding Gp29 (pGp29-3) was isolated from a 48-hour L3 cDNA expression library with a polyclonal rabbit anti-*Brugia malayi* native Gp29. A 32 kD protein was identified on immunoblots containing the pGp29-3 recombinant fusion peptide developed with antibodies against 1) *B. malayi* native Gp29; 2) *B.* pahangi native Gp29; 3) Glutathione S-transferase-*B. malayi* Gp29 fusion protein; or 4) Histidine-*B. malayi* Gp29 fusion protein. The 32 kD fusion protein encoded by pGp29-3 corresponds to the 29 kD native protein fused to the 4 kD Beta-D-Galactosidase peptide encoded by the vector. Sequence analysis of the *D. immitis* Gp29 homolog is in progress.

I 107 SURFACE ANTIGENS OF THE INFECTIVE LARVAE OF *D.VIVIPARUS*, J.Gilleard, C.Britton, J.L.Duncan and A.Tait. Department of Veterinary Parasitology and Wellcome Unit of Molecular Parasitology, University of Glasgow, Scotland, UK.

The bovine lungworm *D.viviparus* induces a highly effective immune response in infected cattle and a vaccine, consisting of radiation attenuated infective larvae, has been successfully used for over 30 years. In spite of this notable success, there is little understanding of natural or vaccine-induced immunity to this parasite. As part of an investigation into the nature of this immune response, the characterisation of surface antigens of the cuticle and sheath of the infective larvae was undertaken. Surface biotinylation of exsheathed larvae failed to label any molecules and only 2 major molecules at approximately 70 and 140kDa were labelled with sheathed larvae. Three monoclonal antibodies were generated against the L_3 cuticular surface and the binding of these was inhibited in the presence of phosphorylcholine. The anti-PC mab BP1 also bound to the surface of the *D.viviparus* L_3 cuticle. However neither BP1 or the three mabs generated here bound to the L_3 cuticular surface of a number of other trichostrongylid nematodes examined. Six monoclonal antibodies were generated against the surface of the L_3 sheath and all of these recognised a 29-40kDa antigen on Western blots of L_3 homogenate. Interestingly these mabs also bound to the sheath surface of 10 other trichostrongylid nematodes examined. The binding of these antibodies to the parasite surface was entirely stage-specific, although they bound to somatic tissues in a non stage-specific manner. Immunogold EM studies have revealed that these antibodies do not bind to the epicuticle but to an outer surface layer.

I 108 CLONING AND CHARACTERIZATION OF MICROFILARIAL SHEATH PROTEINS, J. Hirzmann[1], G. Bardehle[2], H. Christ[1], E.J. Conraths[3], M. Hintz[2], G. Hobom[1], A. Schnaufer[1], B. Schützle[3], S. Stirm[2] and H. Zahner[3], Institut für [1]Mikrobiologie und Molekularbiologie, [2]Biochemie und [3]Parasitologie, Justus-Liebig-Universität, D-6300 Giessen.

In several filarial genera a microfilarial sheath represents the major area of interaction between the parasite larvae and immune reactions of the host. Recently we have isolated and purified the microfilarial sheaths of *Litomosoides carinii*. Partial amino acid sequences of sheath constituents were obtained by N-terminal sequencing of soluble fraction components or proteolytic fragments, separated by reversed-phase HPLC. Using these sequences as a starting point for isolating cDNAs we have now identified two different genes coding for the major structural sheath proteins, named gp22 and shp2. In addition we have isolated and sequenced the shp2 homologous cDNA sequences of two *Brugia species*. Both genes are single copy genes and their transcripts undergo a trans-splicing reaction. The derived proteins are synthesized as preproteins and possess striking repetitive regions. The gp22 protein was further characterized and found to be synthesized at high levels at an early stage of embryonic intrauterine development while gp22 mRNA but no gp22 proteins can be detected already in oocytes. We are presently investigating whether the trans-splicing reaction is directly involved in the regulation of stage specific expression of microfilarial sheath proteins.

I 109 AN *ONCHOCERCA VOLVULUS* CLONE CODING FOR TROPOMYOSIN SELECTED BY ANTISERUM TO INFECTIVE STAGE LARVAE, Rosalind E.Jenkins, Nici Gilvary and Albert E.Bianco, Wellcome Research Centre for Parasitic Infections, Imperial College of Science, Technology and Medicine, London, SW7 2BB, U.K.

An adult female *Onchocerca volvulus* cDNA library, constructed in λgt11, was screened with a rabbit antiserum raised against the infective (L3) stages of the closely related *O. lienalis*. Antiserum affinity purified to the β-galactosidase fusion protein of one selected clone, designated MOV14, reacted with a native antigen of apparent molecular weight 42kD in all life cycle stages and also cross-reacted with antigens from the blackfly vector. The 650bp insert was amplified by the polymerase chain reaction, subcloned into the pCR1000 plasmid and sequenced. The deduced amino acid sequence consists of 129 residues with 93% similarity to *Trichostrongylus colubriformis* tropomyosin, an antigen reported to induce protective immunity against L3's in the guinea pig model for trichostrongylodiasis. Accordingly, MOV14 was subcloned into the expression vector pMALcR1 for use in vaccination trials against the infective larvae and microfilariae of *O. lienalis* in mice. Tropomyosin exists in different isoforms associated with muscle or the cytoskeleton, but preliminary immunoelectron microscopic data indicated an unexpected cuticular localization for the antigen. Since tropomyosin is evolutionarily highly conserved, studies are currently under way to determine whether antibodies/autoantibodies to tropomyosin are present in human onchocerciasis infection sera.

I 110 STRUCTURAL FEATURES OF THE ABA-1 ALLERGEN OF *ASCARIS*, Malcolm W. Kennedy, Alan B. McCruden, Andrew Brass, Alan Cooper, Nicholas C. Price, Sharon M. Kelly, Jacqueline F. Christie, Bryan Dunbar, Heather J. Spence and Joyce Moore, Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden, Glasgow G61 1QH, Scotland, UK.

ABA-1 is a potent allergen of *Ascaris suum/lumbricoides*, and appears to have allergenic homologues in a spectrum of other parasitic nematodes. Part of the sequence of the allergen has previously been obtained by direct N-terminal amino acid analysis and the complete sequence is now known from a cDNA encoding ABA-1. There is evidence from the sequence that ABA-1 contains an internal repeat and may have arisen from a duplication event which must have predated the divergence of ascaridid and filarial nematodes. The protein is predicted to have a helix-turn-helix-loop-helix-turn-helix-loop structure with the two loop regions being disulphide linked. A four helix bundle is a highly stable type of structure and purified ABA-1 was found by Differential Scanning Calorimetry to renature upon cooling after denaturation at 100°C. This analysis also provided evidence that the native protein occurs as a dimer. Circular dichroism confirmed alpha-helix content and protein fluorescence revealed an unusual heterogeneity in the environment of the tryptophan residues. Finally, mass spectrometry indicated that ABA-1 has a mass of 14643.2 ± 1.4 Da, but the cDNA predicts a polypeptide of 15257 Da. It is probable, therefore, that ABA-1 is proteolytically trimmed during post-translational modification, and an appropriate consensus cleavage site has been identified.

I 111 Transglutaminase-catalyzed crosslinking is important for the molting of *Onchocerca volvulus* L3 to L4, [1]Sara Lustigman, [1]Betsy Brotman, [1]Tellervo Huima, [2]Kapil Mehta, and [1]Alfred M. Prince, [1]Department of Virology and Parasitology, Lindsley F. Kimball Research Institute of The New York Blood Center, New York, NY 10021; [2]Department of Medical Oncology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030.

Highly insoluble proteins that are probably crosslinked are very common in the cuticle and epicuticle of filarial parasites and other nematode species. We have investigated the possible involvement of transglutaminase (TGase)-catalyzed reactions in the formation of the cuticle and the development of L4 of *O volvulus* by testing the effect of TGase inhibitors on the survival of L3 and the molting of L3 to L4 in vitro. The larvae were cultured in the presence of a specific pseudosubstrate of TGase, monodansylcadaverine (MDC) or in the presence of an active site inhibitor, cystamine (CS). The viability of the larvae was assessed by MTT staining. None of the inhibitors reduced the viability of both larval stages. However, both MDS and CS, reduced in a time and dose-dependent manner, the molting rate of L3. 100% of molting was inhibited in the presence of 150 μM of MDC or CS. The effect of both inhibitors was critical on day 1 and day 2 in culture. Ultrastructural examination of L3's that did not molt indicated that the new L4 cuticle was synthesized, but there appeared to be no complete separation between the L3 cuticle and the L4 epicuticle. A 45 kDa crossrective protein was identified in L3 and adult worms with a Rabbit anti-mammalian TGase. These studies suggest that the molting and the early development of L4 depend on TGase-catalyzed crosslinking.

I 112 AN ANTIGEN IN *TRICHINELLA SPIRALIS* LARVAE THAT CROSS-REACTS WITH KEYHOLE LIMPET HAEMOCYANIN. Jay Modha, Malcolm W. Kennedy, Huw V. Smith and John R. Kusel, Department of Biochemistry, University of Glasgow, Glasgow, G12 8QQ, UK.

A stage-specific antigen with a native non-reduced $M_r > 200,000$ has been identified by western blotting in homogenates of *Trichinella spiralis* muscle-stage larvae (MSL) using an affinity purified rabbit anti-keyhole limpet haemocyanin (KLH) antibody. The antigen is observable however, as multiple bands of lower molecular weight in SDS-PAGE followed by western blotting under reducing conditions. In competition blots recognition of the antigen in *T. spiralis* MSL by anti-KLH antibody is blocked by KLH, and conversely, *T. spiralis* MSL homogenate blocks the recognition of KLH. However, a rabbit anti-*T. spiralis* MSL serum does not recognize blotted KLH.

In indirect immunofluorescence anti-KLH antibodies bind to the surface of *T. spiralis* MSL, but only after pre-treatment of the larvae with the detergent cetyltrimethylammonium bromide (CTAB). Ultrastructural studies show that CTAB treatment of larvae results in the loss of material from the surface. Moreover, the fluorescent lipid probe octadecyl amino-fluorescein (AF18) did not insert into the surface of these larvae. Hence, it is likely that CTAB removes surface lipid from the larvae, into which the probe AF18 inserts, and exposes the antigen which is underneath.

Also, although the MSL antigen blotted onto nitrocellulose is itself periodate insensitive, treatment of larvae with periodate results in shedding of the larval surface, to which anti-KLH antibodies then bind intensely. These observations are discussed with respect to the nature and location of the larval antigen.

I 113 EXTENSIVE DIVERSITY IN THE SEQUENCE BETWEEN INDIVIDUAL REPEATS OF THE TANDEMLY ARRAYED GENE ENCODING THE ABA-1 ALLERGEN OF *ASCARIS SUUM*. Joyce Moore, Heather J Spence & Malcolm W Kennedy, Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden Road, Glasgow, G61 1QH, UK.

A lambda ZapII cDNA expression library has been constructed from mRNA prepared form *Ascaris suum* infective larvae. Immunoscreening with antibodies against excretory/secretory materials of this developmental stage identified several positive clones. Sequence analysis of these showed that all of them represented different sections of a large transcript encoding the ABA-1 allergen or related proteins, arranged in tandem within a gene designated *aba-1*. Whilst some members of the array encode polypeptides of identical amino acid sequence, some are substantially different, although preliminary data would suggest that they are of the same length and certain amino acid residues are conserved between all repeats so far analysed. The most divergent pair of 'repeat' units show only 49% identity at the amino acid level and 68% similarity when conservative changes are allowed. It is not known if every protein encoded by *aba-1* is allergenic, but it may suggest that the allergen produced by individual parasites is highly heterogeneous.

I 114 CLONING AND IDENTIFICATION OF H11, A HIGHLY PROTECTIVE ANTIGEN FROM *HAEMONCHUS CONTORTUS*, Edward A.Munn, Margaret Graham, Trevor S.Smith[1], Susan E. Newton[2], David Knox, Joanna Oliver[3] and Fiona Smith[4]. [1]AFRC Institute of Animal Physiology and Genetics Research, Cambridge Research Station, Babraham, Cambridge CB2 4AT, U.K.; [2]Pitman-Moore Australia, Molecular Biology Research Unit, Veterinary Clinic Centre University of Melbourne, Werribee, Victoria 3030, Australia; [3]The Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH, U.K; [4]Pitman-Moore Ltd, Breakspear Road South, Harefield, Uxbridge, Middlesex UB9 6LS, U.K.

H11 is an integral membrane glycoprotein present in the intestinal microvillar plasma membrane of the parasitic stages of the nematode *Haemonchus contortus*. It runs on SDS-PAGE, both reduced and non-reduced as a doublet with M_r 110kDa. Although it is a "hidden" antigen, H11, when injected, is very immunogenic. Lambs of three breeds vaccinated with purified H11 develop high levels of circulating antibodies. Judged by reductions in egg output and worm burden, lambs are protected on average by 90% with greater reductions in female worms than males. Level of protection correlates with specific antibody level. Cloned cDNA and PCR products have been sequenced and the deduced amino acid sequence partially confirmed by direct amino acid sequencing of fragments. The putative transmembrane segment and N-glycosylation sites have been identified. The function of the protein deduced by sequence identity has been confirmed by assay and specific inhibitor studies. The activity of the protein is inhibited by serum antibodies from vaccinated animals. Inhibition correlates with degree of protection. Expression of the recombinant antigens in *E.coli* and eukaryotic cells for commercial application is in hand.

I 115 RECOMBINANT ACTIN AND A PUTATIVE CA^{++} BINDING PROTEIN AS CANDIDATES FOR PREPATENT IMMUNODIAGNOSIS OF SCHISTOSOMIASIS MANSONI, Guilherme Oliveira and W. Michael Kemp, Department of Biology, Texas A&M University, College Station, TX 77843

An adult worm cDNA expression library was screened with serum obtained from mice infected for four weeks with *S. mansoni*. Seven positive clones ranging in size from 290 to 1600 bp were isolated, purified, and partly characterized. All of the seven clones have been partly or entirely sequenced, and homologous sequences were sought in GeneBank and EMBL databases. The clones appear unique, except for clone SmAct1 that displayed almost 80% homology to chicken cytoplasmic actin. Another actin clone, SmAct2, was isolated from the cDNA library using a probe prepared from SmAct1, these clones displayed 82% homology at the overlapping regions. Southern Blot analysis of digested adult worm DNA is consistent with the existence of at least two actin genes per haploid genome of *S. mansoni*. Computer analysis of the deduced amino acid sequence of clone 2SAP2 indicated the presence of a putative EF hand motif. The potential Ca^{++} binding site contains all of the conserved amino acid residues characteristic of an EF hand motif. Expression of clone 2SAP2 was obtained with the pFlag vector, yielding a recombinant protein of about 12 kDa. ELISA and dot-blot analysis demonstrated that normal mouse serum had a low level of reactivity against extract of cells expressing 2SAP2, while 8 week, and 4 week infection serum had significantly higher levels of reactivity. The immunoreactivity of the remaining recombinant clones, and their entire cDNA sequences are currently under investigation. (This work was supported by NIH Grant # AI 26505)

I 116 IDENTIFICATION AND CHARACTERIZATION OF RECOMBINANT CLONES REACTIVE WITH A MONOCLONAL ANTIBODY SPECIFIC FOR THE SURFACE OF *BRUGIA MALAYI* INFECTIVE LARVAE.

A.P. Page, M. Philipp*, F. Perler, C. Maina and C. Carlow. New England Biolabs, Beverly MA. 01915. *Tulane Regional Primate Research Center, Covington LA. 70433.

B. malayi infective larvae express a species- and stage-specific surface epitope recognized by an IgM monoclonal antibody (D1E5). This antigen is developmentally regulated, implying an association with the parasites transition from vector to mammalian hosts. Chemical cross-linking of monoclonal antibody/antigen complexes enabled detection of parasite antigen in immunoblots, representing two antigens migrating at approximately 50 and 60 kDa. This crosslinking method was applied to screen genomic expression libraries, and three unique clones were selected. Genomic organization in *B. malayi* was investigated by Southern blot hybridization using the individual inserts as probes, further substantiating the uniqueness of these clones. Sequence analysis of the genomic clones did not reveal any significant protein or DNA sequence homologies. The three inserts were subcloned into appropriate vectors for expression in the maltose binding protein (MBP) and baculovirus expression systems. Subsequent immunizations resulted in only IgM responses, with varying degrees of reactivity to the surface of larvae. The genomic inserts were then used as probes to screen an adult cDNA library. Two independent clones were selected, and sequence analysis is well underway, revealing areas of both homology and uniqueness between the cDNA and genomic clones. These cDNA clones are being further characterized with the goal of identifying this interesting larval surface component.

I 117 VACCINATION OF SHEEP WITH EXCRETED/SECRETED CYSTEINE PROTEASES OF ADULT *FASCIOLA HEPATICA*.

Michael Panaccio, Gene Wijffels, Liliana Salvatore, Marina Dosen, Lachlan Wilson, John Waddington, Jenny Sexton, Jonathan Wicker, Fraser Bowen, Tom Friedel, and Terry W. Spithill. Victorian Institute of Animal Science, Department of Food and Agriculture, Attwood, 3049, Australia and Ciba-Giegy Research Station, Kemp's Creek, Australia.

There has been some evidence from several parasite systems that proteases might have potential as a protective antigen against parasitic infections. A cysteine protease complex identified in the regurgitate of adult *Fasciola hepatica* was examined in this context. The cathepsin L related proteases of M_r 28,000 were purified and are being examined in vaccine trials of sheep infected with liver fluke. Ten animals were immunised with the purified proteases and developed antibodies to the cysteine proteases prior to challenge with *F. hepatica* metacercariae. Infection appeared to cause a boost in antibody response by week 4 into infection, and antibody levels are generally sustained during infection. The cysteine proteases are not novel antigens, since low level antibody titres were also detected in non-immunised controls by late infection. Completion of the trial will indicate changes in worm burden, faecal egg counts and therefore worm fecundity relative to controls.

I 118 PROTECTION OF SHEEP AGAINST *FASCIOLA HEPATICA* INFECTION BY VACCINATION WITH NATIVE AND RECOMBINANT GLUTATHIONE S-TRANSFERASE.

Jim Parsons, Michael Panaccio, Sonia Crameri, Jenny Sexton, Gene Wijffels, Catriona Thompson, Lachlan Wilson, Liliana Salvatore, Noel Campbell, Jonathan Wicker*, Fraser Bowen*, Tom Friedel* and Terry Spithill. Victorian Institute of Animal Science, Department of Food and Agriculture, Attwood, 3049, Australia and *Ciba-Geigy Research Station, Kemp's Creek, 2171, Australia.

Vaccination of sheep against fascioliasis, caused by infection with the ruminant liver fluke *Fasciola hepatica*, has generally proved unsuccessful using crude parasite extracts. Sheep do not develop acquired immunity following a primary infection suggesting that a vaccine strategy aimed at using novel antigens may be necessary to control this disease. In our research for a defined vaccine against *F. hepatica*, we have tested the efficacy of fluke glutathione S-transferase (GST) as a novel antigen in sheep. During the course of a natural infection, sheep do not develop a significant antibody response to fluke GST which comprises a mixture of several proteins of 26-26.5 kD. In several experiments sheep were immunised with native GSTs in Freund's adjuvant using various protocols and the fluke burdens within the liver determined after challenge. All vaccinated sheep showed a similar high total antibody titre to GST. The mean level of protection ranged from 6-57% under different vaccination protocols with some animals showing up to 93% protection. However, some groups of sheep were not protected by GST vaccination suggesting that optimal delivery and formulation of GST needs to be examined. Three cDNA sequences encoding fluke GST have been expressed in *E. coli* and have been used in a recombinant vaccine trial in sheep. Vaccination with two recombinant GSTs elicited a mean level of protection of 43-45%. These results confirm the efficacy of GSTs as immunogens against *F. hepatica* in sheep and suggest that a defined recombinant GST vaccine may be feasible.

I 119 IDENTIFICATION AND CHARACTERISATION OF A MAJOR CYSTEINE PROTEASE SECRETED BY ADULT *FASCIOLA HEPATICA* FLUKES,

Natasha L. Perrett, Bob Thong, Elliott Shaw and Frank Ashall, Biology Department, Imperial College, South Kensington, London SW7 2BB, U.K.

Proteases of different stages of the life cycle of *F.hepatica* were examined using gelatin/ SDS-PAGE and fluorogenic aminomethylcoumarin substrates. Numerous proteases were detected, some of which were specific for certain stages of the parasite's life cycle. In particular, E/S material of mature flukes contained a major protease activity that hydrolysed Z-Phe-Arg-aminomethylcoumarin (Z-Phe-Arg-MCA), and this activity was chosen for further study. Cleavage of Z-Phe-Arg-MCA by E/S material of adult flukes was activated by 2-mercaptoethanol and inhibited by E-64, leupeptin and TLCK, but not by inhibitors of serine, metallo or aspartic proteases, indicating that it is due to one or more cysteine protease. The activity had a pH optimum of about pH 5.5 and was substantially the highest activity detected in E/S material with the substrate examined.

The Z-Phe-Arg-MCA hydrolysing activity was purified from adult *F.hepatica* E/S material by anion-exchange high performance liquid chromatography, which produced a single sharp peak of activity. The molecular mass of the enzyme was estimated to be about 40 kD on the basis of amino acid analysis. The purified enzyme readily hydrolysed substrates containing a hydrophobic amino acid residue at the P2 position, but did not readily cleave substrates containing a basic or non-hydrophobic residue at P2, suggesting that it has some resemblance to cathepsin L of other eukaryotes. The Km for Z-Phe-Arg-MCA hydrolysis was 9.5 uM and the kcat was 1.1 s^{-1}. Hydrolysis of Z-Phe-Arg-MCA by the purified enzyme was inhibited strongly by cysteine protease inhibitors. In particular, Z-Phe-Ala-diazomethyl ketone inhibited the enzyme 50-fold more readily than Z-Ala-Phe-diazomethane, in agreement with the P2 specificity for hydrophobic residues. The cysteine protease cleaved albumin into at least three major fragments and produced a clear band of hydrolysis in gelatin gels. The concentration of the cysteine protease in E/S material was about 7.5 ug/ml.

I 120 A *FASCIOLA HEPATICA* TEGUMENT-SPECIFIC PROTEIN CONTAINS A THIOREDOXIN DOMAIN, Charlene D. Richardson and A. C. Rice-Ficht, Department of Medical Biochemistry and Genetics, College of Medicine, Texas A&M University Health Science Center, College Station, TX 77843

Fasciola hepatica is a hermaphroditic trematode with a wide host range. Commonly known as a liver fluke, it infects a variety of mammals including humans and commercially important livestock. A tegument-specific gene has been isolated from a *Fasciola hepatica* expression library with antiserum raised against the tegument of juvenile as well as adult flukes. DNA sequence analysis has indicated the presence of a highly conserved thioredoxin domain. Thioredoxins are small ubiquitous proteins of about 12kDa which have several disulfide oxidation-reduction functions in both prokaryotic and eukaryotic organisms. The thioredoxin observed in the fluke protein appears to function as one domain occupying 35% of the protein's mass. The reactive protein appears to be restricted to patches of the glycocalyx covering the surface spines only. Recent data indicates that the protein is covalently associated with other tegument macromolecules. Analysis of both cDNA and mRNA has indicated a monomer molecular weight of approximately 36kDa. Immunoblotting of native protein extracted from worm tegument with specific antiserum indicates not only a 36kDa protein but a spectrum of protein sizes extending to 400kDa or larger. Fractionation of tegument extracts through gel filtration and affinity chromatography reveal that the monomer is covalently associated with other large macromolecular complexes. These covalent associations may be important for enzymatic activity or for protein localization.

I 121 STRONGYLOIDES STERCORALIS: COMPUTER ASSISTED 3-D RECONSTRUCTION OF AMPHIDIAL NEURONS. G. Schad, F. Ashton, V. Bhopale, and A. Fine, Dept. of Pathobiology, University of Pennsylvania, Phila, PA 19104. Strongyloides stercoralis, an important nematode parasite of man and animals, has a complex life cycle. The direction of development taken at each of several switch points is presumably mediated by environmental signals. One important switch point directs development from an environmentally resistant, developmentally arrested, infective stage to a developmentally active, maturing parasitic form. Caenorhabditis elegans, a related free-living nematode, has a similar switch point. Given appropriate stimuli, the dauer larva, a resistant resting stage, will resume development and mature. The decision to enter into and to exit from the dauer state is controlled by 4 amphidial chemosensory neurons, as demonstrated by ablation studies (Bargmann and Horvitz, Science 251:1243, 1991). The S. stercoralis infective resting larva, which is similar to the dauer larva of C. elegans, resumes feeding and development on receipt of signals (presumably chemical) from a suitable host. In this stage 12 labial and 4 cephalic sensilla do not open to the environment and, therefore, are probably not chemoreceptors. Therefore, the 13 neurons associated with each amphid are likely to include the relevant receptors. These neurons, along with some of their cell bodies, have been reconstructed from serial sections. We are completing the reconstruction in an attempt to homologize the amphidial neurons of the two species. This should serve as a basis for ablation studies aiming to determine the relationship between amphidial neuron function and parasitic development in S. stercoralis.

I 122 A SPECIES SPECIFIC RECOMBINANT ANTIGEN OF *DICTYOCAULUS VIVIPARUS* WITH SEQUENCE HOMOLOGIES TO MAJOR SPERM PROTEINS OF OTHER NEMATODES, Thomas Schnieder, Institute of Parasitology, School of Veterinary Medicine, W-3000 Hannover 71, FRG

The recombinant *Dictyocaulus viviparus* antigen Dv3-14 proved to be highly specific and sensitive in the ELISA and immunoblot for the serological diagnosis of bovine dictyocaulosis. DNA sequencing revealed that Dv3-14 is 471 bp, of which about 90 % is translated as there is a stop codon at position 424. The calculated molecular mass of the translation product is 15.5 kDa. The nucleotide sequence of Dv3-14 shows homologies with a major sperm protein (MSP) from *Ascaris lumbricoides* var. *suum* (131 matches in 170 positions), MSP clone p3-λ4 (287 matches in 356 positions), MSP clone pcSK10B (289 matches in 356 positions) and MSP pseudogene (128 matches in 164 positions) from *Caenorhabditis elegans* var. Bristol, and with *Onchocerca volvulus* MSP1 (49 matches in 59 positions) and MSP2 (40 matches in 44 positions). Between position 9 and 70 of the deduced 141 amino acid sequence of Dv3-14 84 % homology with *A. suum* MSP and 82 % homology with *C. elegans* MSP3 could be found. Immunoblotting analysis of crude antigen preparations of adult worms and third stage larvae revealed that Dv3-14 is adult stage specific. Triton X-114 fractionation of crude adult worm antigen showed that Dv3-14 is a hydrophilic protein and probably not membrane-bound. Dv3-14 can be demonstrated in supernatants of cultures with adult worms that had been kept in cell culture medium for several days. These results indicate that Dv3-14 is a MSP like protein that elicits a specific immune response in *D. viviparus* infected cattle that can be used for immunodiagnosis. If Dv3-14 is part of the *D. viviparus* reproduction system, it may have potential for chemotherapeutical measures and vaccination.

I 123 BIOCHEMICAL ANALYSIS OF CUTICLE SURFACE MUTANTS OF CAENHORHABDITIS ELEGANS, Michael A. Silverman[1], Mark L. Blaxter[2], Christopher D. Link[1], [1] Department of Biological Sciences, University of Denver, Denver, CO 80208, [2] Wellcome Centre for Parasitic Infections, Department of Biology, Imperial College of Science, Technology and Medicine, London SW7 2BB, UK.

We have identified mutations in six genes (*srf* mutants) that lead to ectopic lectin binding on the surface of *C. elegans*. There are two classes of *srf* mutants: pleiotropic and non-pleiotropic. Nonpleiotropic *srf* animals exhibit only ectopic lectin binding and appear wildtype in all other aspects. The pleiotropic mutant phenotypes include ectopic lectin binding, uncoordinated movement, retarded development time, and defective cell migration and axonal pathfinding. The phenotypes observed may be indicative of a defect in the processes needed for sorting and/or processing of glycosylated molecules.

We are doing a biochemical analysis of *srf* animals to understand the mutant phenotypes. Experiments include surface labeling and analysis of whole animal extracts using lectin and Western blotting techniques. Surface labeling with I^{125} reveals an absence of certain low weight molecular bands and a pattern of complex lipids that differ from wildtype worms. Preliminary experiments using lectin blot analysis with WGA and SBA indicate differences in lectin binding components in *srf* mutants. We have not yet observed any structural defects in the cuticle of the *srf* mutants by electron microscopy.

I 124 MOLECULAR CLONING AND CHARACTERISATION OF AN *ASCARIS* GENE ENCODING THE 14kDa ALLERGEN ABA-1, Heather J. Spence, Joyce Moore & Malcolm W. Kennedy, Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden, Glasgow G61 1QH, U.K.

A 14kDa protein, named ABA-1, is the most abundant protein in the body fluid of adult *Ascaris*, and is released by the tissue-penetrating larval stages of the parasite during culture *in vitro*. Immunological work has shown that ABA-1 is an allergen, suggesting that the IgE mediated hypersensitivity response to ABA-1 may contribute to the pathology of ascariasis. In order to further characterise ABA-1, a cDNA expression library was constructed in λZAPII using mRNA prepared from *A. suum* infective larvae. Screening with polyclonal rabbit antiserum to gel-excised ABA-1 protein led to the isolation of a cDNA clone with a 1255bp insert. DNA sequencing of the entire insert revealed that it consists of two 399bp repeats and one truncated repeat. The putative amino acid sequence derived from each of the repeats was identical to the partial N-terminal amino acid sequence of the native ABA-1 protein, which had previously been derived by direct peptide sequencing. These data could be taken to indicate that ABA-1 is translated as a polyprotein which is then processed into 14kDa monomers.

To allow characterisation of the genomic organisation of the aba-1 gene a genomic library was constructed in λGEM-12 prepared from male adult *A.suum* testes, and was screened with the above cDNA clone. This resulted in the isolation of 4 genomic clones which are currently under characterisation.

I 125 THE EXCRETORY-SECRETORY PRODUCTS OF *TOXOCARA CANIS* CAN SUBSTITUTE FOR LIVE LARVAE IN THE IMMUNE SENSITIZATION OF MICE FOR LIVER TRAPPING. V. Ann Stewart and Robert B. Grieve, Department of Pathology, Colorado State University, Fort Collins, CO 80523.

Mice infected with *Toxocara canis* mount a humoral and cellular immune response against surface and excreted antigens as the larvae migrate sequentially through the liver, the lungs, and then other tissues. Although this immune response does not reduce the ability of subsequently encountered larvae to infect the mice, it does markedly alter the distribution of these larvae within the tissues. Upon subsequent exposure, a significantly increased proportion of larvae remain in the liver and a reduced number of larvae reach the brain. Mice were parenterally sensitized with larval excretory-secretory products, alone and in combination with various adjuvants. Larval numbers in livers and brains two weeks after a challenge dose of 500 infective eggs were compared to those in naive mice and mice sensitized with infective larvae. In C57Bl/10 mice, up to 80% of the liver trapping effect observed with previous exposure to live larvae can be achieved with a combination of larval excretory-secretory products and adjuvant. Sensitization of mice with larval excretory-secretory products significantly affects larval distribution and enhances retention of larvae in the liver.

I 126 EXPRESSION AND CHARACTERISATION OF GP29, A CUTICULAR GLUTATHIONE PEROXIDASE OF FILARIAL PARASITES, Liang Tang, Edith Cookson and Murray E. Selkirk. Department of Biochemistry, Imperial College of Science, Technology & Medicine, London SW7 2AZ, UK.

We have recently cloned and identified the major soluble cuticular protein of adult *Brugia pahangi*, *Brugia malayi* and *Wuchereria bancrofti* as an unusual secretory form of glutathione peroxidase (GSHPx) which shows approximately 43% homology to mammalian cytosolic enzymes. The primary structure of the protein is highly conserved in different species of lymphatic filariae, and only trace amounts of gp29 mRNA are detectable in microfilariae. Subcloning of full length cDNA into an SP6 vector and subsequent translation in vitro indicates that the clone represents a true secretory protein, as it is translocated into pancreatic ER preparations and glycosylated following cleavage of the signal peptide. Gp29 was expressed in insect cells via a baculovirus vector, and the resultant protein was glycosylated, secreted and enzymatically active. Possible biological functions for such an enzyme in filarial cuticle include inhibition of the oxidative burst of host leucocytes, potentially limiting cytotoxic effector mechanisms. Another possibility is that the enzyme catalyses the formation of tyrosine-derived cross-links known to exist in nematode cuticular proteins. We are currently utilising the recombinant protein to investigate these alternatives, and characterising the enzyme in terms of substrate specificity, kinetic properties and inhibitor sensitivity.

I 127 GLUTATHIONE S-TRANSFERASE OF *DIROFILARIA*, Lakshmi Venkatakrishnaiah and Eric R. James, Department of Opthalmology, Medical University of South Carolina, Charleston, SC 29425.

Glutathione S-transferases (GSTs) are a group of enzymes which scavenge hydroperoxide and other electrophilic radicals and may aid in protecting parasites against reactive oxidants produced by metabolism, by oxidant generating drugs and by phagocytic cells of the host. Recombinant GSTs from the trematodes *Schistosoma* and *Fasciola* have been shown to be good protective immunogens. The aim of this study is to obtain recombinant *Dirofilaria* and *Onchocerca* GSTs for use in studies of protective immunity in our rodent onchocerciasis model. To this end two separate tracks are being pursued. The first is to purify GST biochemically and obtain an N-terminal sequence of the protein to design oligonucleotides for screening: Purified GST has been obtained from adult worms of *Dirofilaria immitis* using affinity chromatography and PAGE. The second approach is to utilise PCR to isolate a significant fragment of the gene using degenerate oligonucleotide primer pairs designed to conserved regions of known GSTs and to use this fragment to screen a cDNA library. With *D. immitis* first strand cDNA as template a single 280 bp band was produced. A partial sequence of this product has been obtained but indicates only limited similarity to known GSTs. However, the portion sequenced covers a region of considerable variability between GSTs. This 280 bp product is also being cloned into Bluescript to obtain a complete sequence. Additionally, clones of the *S. japonicum* and *C. elegans* GST cDNAs have been obtained and are being used as probes for screening. (Support from the Edna McConnell Clark Foundation, NIH, the World Bank/UNDP/WHO Special Programme for Research in Tropical Diseases and Research to Prevent Blindness Inc. is acknowledged).

I 128 CHARACTERISATION OF THE EXCRETED/SECRETED CYSTEINE PROTEASES OF ADULT *FASCIOLA HEPATICA*. Gene Wijffels, Michael Panaccio, Liliana Salvatore, Ian D. Walker, and Terry W. Spithill, Victorian Institute of Animal Science, Department of Food Agriculture, Attwood, 3049, Victoria, Australia and the Department of Veterinary Preclinical Sciences, University of Melbourne, Parkville, 3052, Victoria, Australia.

In recent years many studies characterising thiol related proteases and encoding cDNAs have been reported. Generally parasites express several cysteine proteases which are secreted in some cases. These proteases may play a role in parasite invasion, nutrition or evasion of the host immune response. We are investigating the properties of the multiple cysteine proteases produced by *Fasciola hepatica*. Gel analyses of the thiol cathepsin related proteases of adult *F. hepatica* indicated a heterogenous group of proteins in both the whole organism and its regurgitate. The excreted/secreted cysteine proteases have been purified and their enzymatic behaviour is similar to mammalian cathepsin L. Peptide and N-terminal sequence data will confirm their relationship to the cathepsin L family.

I 129 IDENTIFICATION OF NOVEL 3,6-DIDEOXYARABINO-HEXOSE CONTAINING GLYCOPROTEINS IN TRICHINELLA SPIRALIS MUSCLE STAGE LARVAE, N. Wisnewski, M. McNeil, P.J. Brennan, D.L. Wassom, and R.B. Grieve, Department of Pathology, Colorado State University, Fort Collins, CO 80523

The monosaccharide composition of an affinity-purified family of antigenically-related Trichinella spiralis larval glycoproteins was determined by gas chromatography/mass spectrometry. This group of six major glycoproteins, designated TSL-1 (Appleton et al., 1991, Parasit. Today, vol. 7, no. 8:190), originates in the muscle stage (L1) larval stichosome. They are present on the L1 surface and in excretory/secretory products of L1 larvae, are stage-specific, and are highly immunodominant (Denkers et al., 1990, Mol. Biochem. Parasit., 41: 241). The glycosyl composition of TSL-1 was remarkable in two respects: 1) fucose accounted for 36 molar percent of the glycosyl residues; and, 2) a 3,6-dideoxyhexose was identified, which accounted for approximately 24 molar percent of the glycosyl residues. Previously, 3,6-dideoxyhexoses have been found only in certain bacterial lipopolysaccharides and in ascaroside alcohols (ascarylose) of Ascaris eggs. The TSL-1 3,6-dideoxyhexose has been chemically identified as a 3,6-dideoxyarabino-hexose, the same as found in Ascaris eggs. However, the absolute configuration (D or L) has not yet been determined. Methylation analysis indicated that TSL-1 3,6-dideoxyarabino-hexose was present entirely as non-reducing terminal residues. Approximately 80% of the fucose also was present as non-reducing terminal residues, with the remaining fucose being 3,4 linked. It is believed that this is the first report of a 3,6-dideoxyhexose residue found on a glycoprotein.

Physiology, Biochemistry and Molecular Biology

I 200 ARE MITOCHONDRIA INHERITED PATERNALLY IN ASCARIS? Timothy J.C. Anderson[1], Richard Komuniecki[2], Patricia Komuniecki[2] and John Jaenike[1]. [1] Department of Biology, University of Rochester, Rochester NY 14627. [2] Department of Biology, University of Toledo, Toledo, OH 43606.

With very few exceptions, mitochondria appear to be transmitted maternally in most organisms, in part because of the large size of the egg relative to the sperm. The parasitic nematode, Ascaris, is unusual in that it has large amoeboid sperm packed with mitochondria that persist at least for some time after initial penetration. Recently, we have demonstrated that while sperm mitochondria are similar to well characterized anaerobic mitochondria from body wall muscle and are cyanide insensitive, they contain dramatically elevated levels of many of the TCA cycle enzymes. We have hypothesized that these enzymes may represent a "preadaptation" to aerobic larval metabolism once the unembryonated "eggs" leave the host. This suggestive biochemical data and the unusual morphology of Ascaris sperm have lead us to consider the possibility that mitochondrial inheritance in Ascaris may have a significant paternal component. The present study was designed to test this hypothesis. Adult male and female ascarids were obtained from lightly infected pigs and fertilized eggs were collected from the individual females and cultured to the second larval stage. Both adult worms and their progeny were scored for sequence polymorphisms by RFLP analysis of PCR amplified mtDNA. In addition the presence of low levels of paternal mtDNA in the second stage larvae was examined using primer pairs designed to selectively amplify paternal mtDNA.

I 201 CHARACTERIZATION OF A NEW ESTERASE GENE FROM *CAENORHABDITIS ELEGANS*. Martine Arpagaus[1,2], Yann Fedon[1], Xavier Cousin[1,3], Danielle Thierry-Mieg[4] and Jean-Pierre Toutant[1]. Différenciation Cellulaire et Croissance, INRA, 2 Place Viala, 34060 Montpellier (1). Biologie des Invertébrés, INRA, Bd Meilland, 06606 Antibes (2). Neurobiologie, ENS, Rue d'Ulm, 75230 Paris (3). CNRS, Route de Mende, 34033, Montpellier, France (4).

The structure of an esterase gene from *Caenorhabditis elegans* has been determined by comparison of the sequences in genomic and cDNA clones. The gene was mapped close to the center of chromosome V and is therefore distinct from *ges-1* (tip of Ch. V) which codes for the gut-specific esterase characterized by McGhee and colleagues (a). It possessed 7 short introns interrupting the coding sequence. The 5' splice site of intron 3 presented the sequence GC instead of the usual GT that was found in the other six introns. The cDNA was trans-spliced with the short leader SL1. The open reading frame indicated that a protein of 557 aminoacids was encoded. The deduced aminoacid sequence did not present a N-terminal signal peptide but a potential N-myristoylation site (GXXXS) provided that the initiator methionine was removed. This protein should therefore remain intracellular. The 200 first N-terminal aminoacids presented a high percentage of identity with members of the esterases family (maximum of 43% with *Torpedo* acetylcholinesterase and lepidopteran juvenile hormone esterase). Percentages of homology dropped to 25-30% when the whole sequences were compared. The active serine was identified as serine 208 in the sequence GQSAG (consensus sequence of esterase: GXSXG). These results as well as the analysis of the secondary structure features of the protein showed that it belongs to the subgroup of esterases in the α/β hydrolase fold family (b).

a. McGhee and Cottrell, 1986, Mol. Gen. Genet. 202, 30-34; McGhee et al., 1990, Genetics 125, 505-514.
b. Ollis et al, 1992, Protein Engeneering, in press.

I 202 GLUCOSE METABOLISM IN THE FILARIAL NEMATODE *ONCHOCERCA GIBSONI*: A C-13 NMR STUDY, Carolyn A. Behm, Division of Biochemistry and Molecular Biology, School of Life Sciences, Australian National University, GPO Box 4, Canberra, Australia

Live adult female parasites of *O. gibsoni* were freed aseptically from their nodules by collagenase digestion and incubated in an NMR tube inside the magnet of a Varian VXR300 NMR spectrometer. The parasites were constantly perfused, in recirculating mode, with a buffered salt solution containing 10mM U-^{13}C-glucose or 1-^{13}C-glucose. Sequential ^{13}C spectra of the living worms were acquired at 90 min intervals during a period of 20 hours and ^{31}P spectra were acquired at the beginning and end of each experiment to monitor the viability of the parasites. At the end of each experiment, high-resolution ^{13}C NMR spectra were also acquired of the incubation medium and of perchloric acid extracts prepared from the parasites.

Under these conditions the parasites metabolised glucose to lactate (the major end-product), HCO_3^- and the minor and variable end-products succinate, acetate, ethanol and glycerol. Glucose was also incorporated into the disaccharide trehalose and into glycogen. Exposure of the parasites for the duration of the experiment to an inhibitor of the enzyme trehalase caused accumulation of trehalose such that significant quantities were released into the incubation medium. The treatment did not affect metabolism of the exogenous glucose to the metabolic end-products listed above.

I 203 TRANSCRIPTIONAL REGULATION OF HOUSEKEEPING GENES IN *C. ELEGANS*, David McK. Bird Department of Nematology and Graduate Genetics Group, University of California, Riverside, CA 92521.

I have been using a number of approaches to study transcriptional regulation of *ama-1 IV*. This *C. elegans* gene encodes the largest subunit of RNA polymerase II and is likely to represent a "typical" housekeeping function.

To identify the *ama-1* cap site, 5'-RACE-PCR was performed. After 70 rounds of amplification, the single resultant product was cloned and sequenced. All of the 27 recombinants examined revealed the presence of *trans*-spliced SL-1. A primer spanning the genomic location of the *trans*-splice junction was synthesized and used in 5'-RACE-PCR to amplify the primary transcript. A major product of ~550 bp was generated, plus a number of larger, minor products which currently are being characterized.

Since coding and regulatory sequences are generally highly conserved between the related nematodes *C. elegans* and *C. briggsae*, a second strategy has been to examine sequences upstream of the *C. briggsae* homologue of *ama-1*. This gene, called *ama-1(rv1)*, was isolated from a *C. briggsae* genomic library (constructed by Terry Snutch and kindly supplied by Dave Baillie) by low stringency with a probe derived from the 5'-end of *ama-1* and extending to within 438 bp of the ATG

Comparison of the inferred amino acids of *ama-1* and *rv1* shows a high degree of similarity and introns occur at identical positions in each gene. Outside the coding region however, the sequences are highly divergent, except for several repeats of a ATTTC motif, phased on the same side of the helix. Ongoing *in vitro* mutation experiments will reveal if these elements play a functional role.

I 204 **EFFECTS OF CYCLOSPORIN A ON *SCHISTOSOMA* AND *FASCIOLA IN VIVO AND IN VITRO*: MODE OF ACTION**, Leslie H Chappell, Jian Fa Liu and Michael C Penlington, Department of Zoology, University of Aberdeen, Aberdeen, AB9 2TN, UK

Cyclosporin A (CsA) is a potent immuno-suppressant drug, with wide clinical application; enigmatically, it also exhibits powerful but selective anti-parasite activity. CsA kills *Schistosoma mansoni* and *Fasciola hepatica in vivo*, dependent upon drug dose, route and timing of administration and age of worms. Its mode of anthelmintic action is not understood but it has been proposed that the drug may augment the immune response. *In vitro* studies, using native CsA and microsome-derived metabolites, suggest, in fact, a direct mode of action, unrelated to immuno-modulation. Exposure of schistosomes and *Fasciola* to CsA and metabolites *in vitro* induces the formation of tegumental and gut lesions. Worms are killed at rates relative to drug concentration and worm age; younger worms are more susceptible and are killed at lower drug doses. Thus CsA alters parasite surface physiology, disrupting ion and nutrient fluxes, and down-regulating energy metabolism. Drug action may be mediated through interaction with cyclophilin-calcineurin complexes, altering phosphatase activity. Both acid and alkaline phosphatase activities are reduced in worms exposed to CsA and the kinetics of glucose transport are modified. The unexpected anthelmintic action of CsA, and its prophylactic properties against *S mansoni in vivo*, argue compellingly that a non-immunosuppressive analogue of this drug could play a valuable role in the control of selected helminth parasites.

I 205 CLONING AND CHARACTERISATION OF A *TRICHINELLA SPIRALIS* MYOD HOMOLOGUE, Bernadette Connolly, Murray E. Selkirk and Deborah F. Smith, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7 2AZ, U.K.

The first stage larva of the nematode *Trichinella spiralis* is an intracellular parasite of mammalian skeletal muscle. The infected muscle cell undergoes profound changes so that within 20 days post infection it is no longer recognisable as a muscle cell. Among other morphological changes there is a loss of striations which reflects, at the biochemical level, a complete loss of muscle specific proteins. This has led to the suggestion that infection by *Trichinella* results in the de-differentiation of the muscle cell. Our current understanding of muscle development and maintenance of the differentiated state in mammalian cells implicates a family of myogenic regulatory proteins, of which MyoD is a member, in this process. One possibility for the molecular mechanism of *Trichinella*-induced de-differentiation is that parasite-encoded proteins which are members of this family may interfere with the normal regulation of the endogenous muscle genes. Using appropriate oligonucleotide primers and PCR we have amplified a 117 bp sequence from *Trichinella* genomic DNA which shows strong homology to MyoD (34/39 amino acids). This sequence has been used to identify a 2.5 kb genomic clone from a phage library. The analysis and characterisation of this clone at the DNA and protein level will be presented.

I 206 CHARACTERIZATION AND ATTEMPTS TO EXPRESSION CLONE AN AVERMECTIN AND GLUTAMATE-SENSITIVE CHLORIDE CHANNEL FROM *CAENORHABDITIS ELEGANS*, D.F. Cully, P.S. Paress, K.K. Liu, and J.P. Arena, Merck Research Laboratories, Rahway, NJ 07065.

The family of compounds called avermectins (AVM) have potent anthelmintic and insecticide activity. *Caenorhabditis elegans*, a soil nematode, has been used to study the mechanism of action of the AVMs. Using the *Xenopus* oocyte as a surrogate expression system for *C. elegans* mRNA, we have identified a *C. elegans* encoded chloride channel that is sensitive to both AVM and glutamate. These responses are blocked by picrotoxin but are insensitive to the neurotransmitter GABA. Size fractionation of *C. elegans* mRNA on agarose gels shows that RNA encoding the AVM and glutamate-sensitive channel is found in the 1.8-2.0 Kb mRNA class. Very small amounts of this purified mRNA (0.9 ng per oocyte) is sufficient to elicit AVM and glutamate responses in oocytes. A cDNA library was synthesized from this active mRNA fraction by using a protocol that enables unidirectional synthesis and cloning of cDNA. We have constructed an expression library containing 80% cDNA inserts with an average size of 1.2 Kb. We have further enriched this library for clones containing full length cDNAs by size separation of linear DNA isolated from large pools of clones, with subsequent religation and transformation. Synthetic RNA has been synthesized from pools of recombinant cDNAs and coinjected into *Xenopus* oocytes with *in vitro* RNA synthesized from a potassium channel gene as a control. We are currently analyzing these recombinants for expression of the AVM and glutamate sensitive channel.

I 207 **DEMONSTRATION OF BOMBESIN-LIKE IMMUNOREACTIVITY AND BOMBESIN/GASTRIN-RELEASING PEPTIDE BINDING SITES IN NEMATODES** M.K.Huntington[1], J.Leykum[2], C.D.Mackenzie[3], T.G.Geary[4], J.F.Williams[1], Departments of Microbiology[1], Biochemistry[2], and Pathology[3], Michigan State University, East Lansing, MI, 48824; and Parasitology Research Group[4], The Upjohn Company, Kalamazoo, MI, 49002

Bombesin-like peptides are biologically active in a wide range of organisms, both vertebrate and invertebrate. We have identified and quantitated bombesin-like immunoreactivity in nematodes of the *Panagrellus, Haemonchus, Ascaris, Onchocerca,* and *Dirofilaria* genera. Detected levels correlated to approximately 500 pg bombesin/g extract. Immunohistology demonstrated that the highest reactivity is found at the hypodermocuticular junction zone. Employing multi-step high pressure liquid chromatography and fast-atom bombardment mass spectroscopy, we have identified the source of the immunoreactivity from *Panagrellus redivivus* as a molecule of 1417.1 molecular weight. Edman degradation revealed a protected amino terminal; efforts to obtain a complete sequence are currently underway.

Receptor-binding assays indicate that specific binding of ^{125}I-labelled gastrin-releasing peptide occurs in cell membrane preparations of *Ascaris* body wall. These binding sites exhibit a K_D of 2.8 nM and a capacity of approximately 1 pmol/g protein in our preparations. Autoradiomicrography demonstrated that this binding is localized along the body wall muscle cell membranes and the hypodermis.

Bombesin has been implicated in the regulation of cell membrane potential, resistance, and ion transport in other organisms. An understanding of the role of bombesin in helminths may therefore provide unique insight into their physiology and an opportunity for targetted selection of broad-spectrum anthelmintic agents directed against this peptide/receptor system.

I 208 PROPERTIES OF AN ENKEPHALINASE-LIKE ENDOPEPTIDASE FROM *CAENORHABDITIS ELEGANS*, R.Elwyn Isaac, Richard Priestly, Linda Eaves and Donald L.Lee, Department of Pure & Applied Biology, University of Leeds, LS2 9JT, U.K.

Petidases, that are responsible for the inactivation of neuropeptide signals at synapses and the clearance of peptide hormones from the body, are attracting great interest, especially for the design of inhibitors of clinical importance. These neuropeptide-degrading enzymes have a broad substrate specificity and are widely distributed in mammalian tissues. The discovery and design of potent selective inhibitors of peptidases has provided powerful tools to investigate the involvement of individual enzymes in the metabolism and inactivation of neuropeptides.

Enkephalinase is a well characterised cell-surface peptidase that is partly responsible for the inactivation of enkephalins in mammalian brain. It's precise name is endopeptidase 24.11 and it is a member of the Zn-metalloendopeptidase family and is selectively inhibited by phosphoramidon.

We have now shown that membranes prepared from *Caenorhabditis elegans* are able to degrade enkephalin analogues at the Gly-Phe bond and that this activity is sensitive to inhibition by 10 μM-phosphoramidon. This 'enkephalinase' activity has been characterised and we have measured its activity in membranes prepared from different stages of *C.elegans* development. We intend to use molecular approaches to probe the functions of this neuropeptide-degrading enzyme in this nematode.

I 209 **AMPLIFICATION OF *D. IMMITIS* cDNA WITH AN *O. VOLVULUS* SPLICED LEADER PRIMER IDENTIFIES TWO PUTATIVE Cu/Zn SUPEROXIDE DISMUTASE cDNAs,** Eric R. James, David L. McLean, Francine B. Perler* and Larry A. McReynolds* Department of Ophthalmology, Medical University of South Carolina, Charleston, SC, 29425 and *New England Biolabs, Beverly, MA, 01915.

Degenerate oligonucleotide primers designed to conserved regions of Cu/Zn superoxide dismutase (SOD) cDNAs were used in a polymerase chain reaction (PCR) with *D. immitis* cDNA as template. A single 210bp product resulted which yielded a sequence 82% similar to an analogous region of the described *O. volvulus* Cu/Zn SOD (OvSOD). A specific antisense 23mer primer to the central portion of this sequence, which also had considerable similarity to all other Cu/Zn SODs including 71% and 88% respectively to human extracellular SOD (ECSOD) and *S. mansoni* signal peptide SOD (SmSPSOD), was used in combination with an *O. volvulus* spliced leader (OVSL) primer in a PCR (45°C anealing temperature) with *D. immitis* adult worm first strand cDNA as template. Two bands of approximately 300bp and 400bp were obtained. Sequences of both products were consistent with Cu/Zn SODs. The 300bp product was 85% similar to the OvSOD and identical in the overlapping region to the original 210bp product. The 400bp product was 67% similar to both the OvSOD and the 300bp product. The OVSL primer sequences were identified in both 300 and 400bp products. In the 300bp product a 21nt sequence occurred between the OVSL and start ATG which was 67% similar to the comparable OvSOD 21nt sequence. A 111nt region immediately 3' to the OVSL in the 400bp product coded for a significantly hydrophobic peptide suggestive of a signal/leader domain. This putative leader sequence has some similarities to the 5' leader sequences of both human ECSOD and SmSPSOD. We estimate approximately 250bp of the 3' regions of both putative SODs remain to be sequenced. To date, sequences of gel purified PCR products generated using sense primers with a dT primer or a terminal 3' antisense OvSOD primer have been ambiguous, at least indicating both cDNAs are of similar size 3'. PCR protocols and primers are being varied to resolve the 3' regions for both cDNAs and the 300 and 400bp products are also being used to screen a *D. immitis* cDNA library. [Support from NIH (EY07542), The Clark Foundation, World Bank/ UNDP/WHO Special Programme for Research in Tropical Diseases and Research to Prevent Blindness Inc. is acknowledged].

I 210 EFFECT OF SELECTION FOR BENZIMIDAZOLE RESISTANCE IN *HAEMONCHUS CONTORTUS* ON ß-TUBULIN ISOTYPE 1 AND ISOTYPE 2 GENES

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University of Utrecht, The Netherlands

In vitro selection for benzimidazole (BZ) resistance in the nematode *Haemonchus contortus*, a parasite of sheep, indicated the involvement of ß-tubulin genes in the mechanism governing BZ resistance. Two distinct ß-tubulin isotype classes were analyzed. Selection with increasing concentrations thiabendazole (TBZ) showed that increasing BZ resistance might correlate to a two step adaptation mechanism: initially, at lower degrees of developing resistance, selection for a single ß-tubulin isotype 1 variant takes place, followed at higher degrees of drugresistance, by an abrupt elimination of individuals carrying ß-tubulin isotype 2 genes in their genome. A survey of resistant populations isolated from the field indicated that in one population (RNO) the same mechanism was found, while in other resistant populations isotype 2 was still present. In order to asses that these results were not due to accidental loss of non functional isotype 2 genes, RNA was prepared from eggs, L3 larvae and adults from a susceptible (SE) population and the in vitro selected (RE4) population with the highest degree of resistance and quantitated in a Northern blot. In population SE mRNA from both isotypes was present in all developmental stages tested, while in RE4 only mRNA from isotype 1 was found.

I 211 RETINOL BINDING PROTEIN (RBP) OF *ONCHOCERCA* MAY NOT CROSS-REACT WITH HUMAN RBP, Preeti Gupta Lal and Eric R. James, Department of Ophthalmology, Medical University of South Carolina, Charleston, SC, 29425

The concentration of retinol in adult *Onchocerca volvulus* may be up to 8 times higher than that of the surrounding host tissues (Sturchler et al, Acta Trop. 1983, 40:261) indicating that these parasites absorb and concentrate vitamin A. In the absence of visual function, retinoids present in the worms are likely to be utilized to support growth, differentiation and reproduction. Retinol is strongly lipophylic and requires retinol binding proteins (RBPs) to facilitate transport through aqueous environments. A high retinol concentration implies a high RBP content. Autoimmunity has been suggested to be a component of onchocercal ocular pathology and human interphotoreceptor RBP (IRBP) is autoimmunogenic. The purpose of our study is to attempt to identify, clone and sequence parasite protein(s) which may cross-react with human RBPs. A rabbit polyclonal antiserum to native bovine IRBP was raised and used both in ELISA and in Western blots against adult *O volvulus* soluble homogenate. By ELISA the antibody recognised several proteins. We were able to identify one major band of approximately 18 kDa in Western blots which is being further purified biochemically. We have no evidence yet that this 18 kDa protein is a parasite RBP. The anti-IRBP antiserum was used to screen a λgt11 *O. volvulus* cDNA library, however, screening of approximately one million plaques has yielded no positive clones so far. A second rabbit antiserum to denatured IRBP is currently being raised for screening studies. We are also assembling clones of bovine IRBP, rat cellular RBP (CRBPI) and rat CRBPI I for genomic and cDNA screening. To date Southern blots of *O volvulus* genomic DNA probed with the rat CRBPI clone have yielded negative results. [Support from NIH (EY 06462) and Research to Prevent Blindness Inc. is acknowledged].

I 212 AMINO-ACID RECEPTOR SUBUNITS CLONED FROM THE PARASITIC NEMATODE *HAEMONCHUS CONTORTUS*, D.Laughton, A.Wolstenholme, P. Harris, P.Towner, and G.Lunt, Dept. of Biochemistry, University of Bath, Bath BA2 7AY, UK.

The study of the molecular structure of nematode neuroreceptors is interesting both from an evolutionary aspect, and because of their potential as targets for novel anthelminthics. Using a combination of RACE and Inverse PCR, one full length cDNA (HG10) and one partial cDNA (HG5), encoding putative subunits of an inhibitory amino-acid receptor, were isolated from a mixed egg stage of the parasitic nematode, *Haemonchus contortus*. The two subunits share approximately 35% amino acid identity with each other, and 35% identity with other previously cloned vertebrate and invertebrate GABA and Glycine receptor subunits. Similarities to other members of the Ligand-Gated Receptor Superfamily include the four membrane spanning domains and an extended extracellular N-terminus, which contains a conserved 16 amino acid stretch bounded by two cysteine residues (the "cysteine loop"). Expression studies with HG10 in *Xenopus* oocytes are in progress.

I 213 A FAMILY OF PUTATIVE CHEMOSENSORY RECEPTOR GENES IN FREE-LIVING AND PARASITIC NEMATODES. Leo X. Liu, Infectious Diseases Division, Harvard Medical School, Boston, MA and Department of Biology, Imperial College, London, UK.

Chemosensation of environmental signals by an organism represents a critical initial step leading to chemotaxis, feeding, reproduction, and other behavioral and developmental processes. For a free-living nematode such as *Caenorhabditis elegans*, such environmental cues include simple chemoattractants, bacterial food signals, and the dauer pheromone. For a parasitic nematode, however, these signals are likely to include host-specific factors which will influence its host finding, anatomic localization within the host, and development to subsequent stages. A large multigene family encoding putative odorant receptors has been isolated from mammalian olfactory tissue. Some members of this G protein-coupled receptor subfamily are also expressed in spermatozoa, suggesting that they are involved in chemosensory and chemotactic responses. Using a PCR homology cloning strategy, a set of degenerate oligonucleotides corresponding to selected conserved regions of transmembrane domains 2, 3, 6 and 7 of the putative odorant receptor family were used in the PCR to amplify first strand cDNA prepared from poly A(+) RNA isolated from *C. elegans* and the parasitic filarial nematode *Brugia malayi*. Appropriately sized PCR products were examined by restriction endonuclease digestion, and those products containing multiple DNA species (indicative of a multigene family) were subcloned and sequenced. All of the nematode partial cDNA sequences obtained to date are novel and reveal sequence motifs typical of G protein-coupled receptors, including hydrophobic putative transmembrane domains. The nematode sequences share a number of conserved residues with known mammalian odorant receptor sequences, but also share a number of distinct sequence motifs with one another, suggesting that they represent a phylogenetically and/or functionally distinct subfamily of chemosensory receptors. Nematode cDNA and genomic DNA libraries are currently being screened in order to obtain full length DNA sequences, and physical mapping of these genes is also being performed by probing an ordered *C. elegans* YAC library. These results indicate that nematodes possess a novel gene family of putative chemosensory receptors. Further study of these chemosensory receptors in parasitic nematodes may suggest avenues for pharmacologic or immunoprophylactic interventions at this critical host-parasite interface.

I 214 N-ACETYLATION OF 5-HT IS A MAJOR METABOLIC PATHWAY IN NEMATODES AND IS DEVELOPMENTALLY REGULATED IN *C.ELEGANS*, R.Muimo and R.E.Isaac, Department of Pure and Applied Biology, University of Leeds, Leeds, U.K.

Biogenic amines, such as 5-HT, octopamine and dopamine, function as neurotransmitters and/or hormones in nematodes. 5-HT and octopamine are involved in the regulation of both feeding and reproduction in *Caenorhabditis elegans*. The NSM neurons innervating the pharynx and the HSN cells that synapse with the sex muscles of adult hermaphrodite *C.elegans* contain 5-HT. Treating the nematode with 5-HT increases the rate of pharyngeal pumping, stimulates egg-laying and influences locomotor activity. Octopamine acts antagonistically to 5-HT by decreasing pharyngeal pumping and inhibiting egg-laying.
Hormone and neurotransmitter levels can be regulated by metabolic inactivation and we have shown that parasitic nematodes have high levels of N-acetyltransferase (NAT) activity and that N-acetylation could be a primary route of amine inactivation in nematodes. NAT has now been purified from *Ascaridia galli* and we report some of the properties of this enzyme. Of particular interest is the ability of NAT to N-acetylate arylalkyl-, phenyl- and catechol-amines and the observation that most of the enzyme activity in adult female worms is associated with the gonads. N-acetylation of 5-HT is also the only reaction detected in *C.elegans* tissues. The enzyme is a soluble protein that has an apparent Km of 30μM for 5-HT. The specific activity of the C.elegans NAT increases exponentially with development from L1 through to adult hermaphrodites. This developmental profile correlates temporally with the appearance of 5-HT in the HSN neurons and the rise in the octopamine content of the worm at the adult stage.

I 215 PHOTOAFFINITY LABELLING OF BENZIMIDAZOLE BINDING SITES FROM *HAEMONCHUS CONTORTUS* WITH A PHOTOACTIVE ANALOGUE OF FENBENDAZOLE.
Bakela Nare, Roger K. Prichard and Elias Georges, Institute of Parasitology, McGill University, Ste-Anne-de-Bellevue, Quebec, H9X 3V9, Canada.
Benzimidazole (BZ) carbamates are a class of broad-spectrum anthelmintics whose mode of action is thought to result from the disruption of parasite tubulin-microtubule equilibrium. The widespread BZ-resistance in parasitic nematodes following regular treatment with BZ may be caused by a number of cellular alterations. One such change may be in the tubulin genes, which affects BZ binding. Previous *in vitro* drug binding studies using ^{3}H-BZ demonstrated a reduction in binding affinity in BZ-resistant *Haemonchus contortus*. However, identification and characterisation of BZ binding site(s) in parasites has been limited by the low concentration of BZ-binding proteins and the instability of the BZ-receptor complex. We have synthesised a photoactive BZ analogue (methyl-5-substitute azidosalicylido-phenoxybenzimidazole-2-yl carbamate; azido-BZ) by reacting an amino-fenbendazole derivative with N-hydroxysuccinimidyl-4-azidosalicylate. The product was iodinated and purified by HPLC. Incubation of ^{125}I-azido-BZ with cytosolic fractions from *H. contortus* followed by UV irradiation resulted in covalent crosslinking of a 54 kDa protein as demonstrated by SDS-PAGE and autoradiography. The specificity of azido-BZ towards its receptor protein was demonstrated in a UV crosslinking assay using unmodified amino-fenbendazole as well as other structural analogues of BZ. Azido-BZ specifically labelled a similar polypeptide in ovine brain cytosol. In both nematode and brain cytosolic supernatants, the labelled protein co-migrated with purified tubulin on SDS-PAGE. It appears that azido-BZ specifically photolabels tubulin; the first direct evidence that BZs specifically bind to tubulin. This BZ analogue will be a valuable tool in the characterisation of BZ receptors and any molecular changes that may be associated with the development of BZ resistance in parasitic nematodes. (Supported by NSERC strategic grant # STR GP230).

I 216 NEUTRAL GLYCOSPHINGOLIPIDS OF *ECHINOCOCCUS MULTILOCULARIS* METACESTODES, Florence Persat+, Jean-François Bouhours§, Claude Vincent*, Anne-Françoise Petavy+ and Madeleine Mojon+, +Département de Parasitologie et Pathologie Exotique, Université Claude Bernard, 8 Avenue Rockefeller 69373 Lyon, France, §Institut National de la Santé et de la Recherche Médicale, Unité 76, Paris, France, and *Institut National de la Santé et de la Recherche Médicale, Unité 80, Lyon, France.
Neutral glycosphingolipids of the metacestodes of *Echinococcus multilocularis* were resolved by high performance thin layer chromatography into 12 fractions. Nine of these fractions were permethylated, analyzed by electron impact-mass spectrometry and submitted to methylation analysis by gas chromatography-mass spectrometry. Native fractions were analyzed by liquid secondary ion-mass spectrometry and degraded sequentially by exoglycosidases. In addition to a previously described galactosylceramide (1), a di-, a tri- and a tetragalactosyceramide having Galβ1-6Gal internal linkages were characterized (2). This type of carbohydrate chain has been described in glycolipids of a marine mollusc, *Turbo cornutus* (3). In addition 2 novel fucolipids were found with the following structures : Fucα1-3Galβ1-6Gal-Cer and Galβ1-6(Fucα1-3)Galβ1-6Gal-Cer. Ceramides contained sphinganine and either nonhydroxy fatty acids with 16, 18, 26 and 28 carbon atoms, or hydroxy fatty acids, with 16 and 18 carbon atoms. Di-, tri- and tetragalactosylceramides containing the Galβ1-6Gal disaccharide, were found immunogenic in humans.
1) Persat F. *et al.* (1990) *Mol. Biochem. Parasitol.*, **41**, 1-6
2) Persat F. *et al.* (1992) *J. Biol. Chem.*, **267**, 8764-8769
3) Matsubara T. and Hayashi A. (1981) *J. Biochem.*, **89**, 645-650

I 217 REVERSE GENETICS OF *C. elegans*, USING THE Tc1 TRANSPOSON, Ronald H.A. Plasterk, José T.M. Groenen, Joyce van Meurs, A. Broeks, R. Zwaal, S. Youngman, Department of Molecular Biology, Netherlands Cancer Institute, Plesmanlaan 121, 1066CX Amsterdam, the Netherlands, E-mail RPLAS@NKI.NL.
We established a library of 5000 frozen cultures of the nematode *C. elegans* mutagenized by insertion of Tc1 transposons. Each culture was initiated by less than 10 animals of mixed age; after thawing each culture yields 100-1000 living animals. This is sufficient to ensure the presence of insertions in most genes. DNA from part of each culture DNA is available for analysis by PCR, using one Tc1- and one gene-specific primer. Insertion sites are determined by direct sequencing of PCR products; interesting mutant animals can be recovered. Knowing the sequence of (parts of) the *C. elegans* genome, one can now obtain a transposon insertion derivative of a gene within a few days. This makes it relatively easy to go from DNA sequence to phenotype. Examples of this approach will be described (a.o. for genes encoding G-protein α subunits, and P-glycoproteins).
Transposon insertions can be taken as starting points for the introduction of further alterations into the genome (site specific mutations or deletions).

I 218 IDENTIFICATION OF STEROID HORMONE RECEPTORS IN *DIROFILARIA IMMITIS*, Jennifer Richer and Claude V. Maina, New England Biolabs, Beverly, MA 01915

In order to understand developmental regulation in *D. immitis*, we have begun to study the genetic regulation of molting. We are examining the potential role that steroid hormone receptors, especially the ecdysone hormone receptor, play in molting.

The presence, although not the synthesis, of ecdysone in *D. immitis* has been demonstrated by a variety of methods. Ecdysone has been implicated in the L3 to L4 molt and in the formation of microfilaria. We are using the Drosophila ecdysone receptor (EcR), a member of the steroid hormone superfamily, as a means to identify the potential *D. immitis* homolog as well as to identify other steroid hormone receptors of the filarial parasite.

In Southern blot experiments, EcR cDNA shows strong hybridization, at high stringency, to a specific *D. immitis* genomic DNA fragment. This hybridization localizes to the region of EcR that contains both the hormone and DNA binding domains. Experiments are underway to further localize the hybridizing region as well as clone the hybridizing *D. immitis* DNA fragment.

In immuno-blot experiments, anti-EcR polyclonal sera reacts with a 65 kd protein present in adult female *D. immitis* and possibly in L3's, but not in adult males, mf or L4's. A monoclonal antibody that maps to the DNA binding domain of the Drosophila EcR reacts with a 35 kd protein present in adult males, adult females and L4's, but not in mf or L3's. Both the 35 kd and 65 kd proteins have been cloned from an adult female cDNA library and are being sequenced.

It is our hope that this approach will advance our understanding of developmental regulation in filarial parasites.

I 219 THE *unc-52* GENE OF *Caenorhabditis elegans* ENCODES A PROTEOGLYCAN COMPONENT OF THE BASEMENT MEMBRANE, Teresa M. Rogalski, Erin J. Gilchrist, Gregory P. Mullen and Donald G. Moerman, Department of Zoology, University of British Columbia, Vancouver, B.C. Canada V6T 1Z4

Mutations in the *unc-52* gene of *C. elegans* affect the attachment of the myofilament lattice to the muscle cell membrane. We have sequenced the *unc-52* gene which spans almost 15 kb of genomic DNA and consists of 26 exons. The longest open reading frame of this gene encodes a 2,481 amino acid protein that is homologous to the core protein of the mammalian basement membrane heparan sulfate proteoglycan (Noonan et al., J. Biol. Chem. 266:22939-22947, 1991). The nematode proteoglycan has a signal peptide and four distinct domains. The first domain is unique to the *unc*-52 polypeptide whereas the three remaining domains contain sequences found in other proteins including the LDL-receptor (Domain II), laminin (Domain III) and N-CAM (Domain IV). We have identified three alternatively spliced transcripts that encode different C-terminal amino acid sequences. The two larger transcripts encode proteins containing all or part of Domain IV, whereas the smaller transcript encodes a shortened polypeptide that completely lacks Domain IV. We have identified the sequence alterations for three *unc-52* mutant alleles, and in each case, the disorganized muscle phenotype produced by the mutation is due to a defect in Domain IV. We have also mapped the epitopes recognized by two monoclonal antibodies to a region in Domain IV of the *unc-52* encoded protein sequence. These antibodies show staining of the basement membrane at muscle/ muscle cell boundaries and at the muscle/ hypodermal interface in adult hermaphrodites (Francis and Waterston, J. Cell Biol. 114:465-479, 1991).

I 220 BENZIMIDAZOLE RESISTANCE IN THE PARASITIC NEMATODE HAEMONCHUS CONTORTUS: THE ROLE OF β-TUBULIN, Marleen H. Roos, Marcel S.G. Kwa, Jetty G. Veenstra and Jaap H. Boersema, Department of Parasitology, Faculty of Veterinary Medicin, University of Utrecht, Utrecht, The Netherlands.

Resistance to benzimidazoles (BZ), a group of antimitotic drugs that is widely used against parasitic nematodes of man and animals, is emerging rapidly in Haemonchus contortus, a sheep parasite. We investigated the molecular mechanism of this resistance. Our previous studies indicated that changes in β-tubulin isotype 1 genes and/or closely linked factor(s) could be involved. To discriminate between these possibilities we determined the complete genomic sequence from a β-tubulin gene, that we cloned from population Resistant Utrecht (RU), gru-1. The gene contained 10 exons and 9 introns. A detailed RFLP study was carried out using restriction sites selected from this sequence. The results indicate that susceptible parasite populations contain at least three β-tubulin isotype 1 variant genes. Five of the six resistant populations, including RU, contain only one variant and in one other resistant population, two variants were found. The apparently selected genes were probably functional because they were transcribed and processed to mRNA using the nematode specific trans-sliced leader (SL1) and a splice acceptor site 26 bp upstream from the start codon. Comparison of the derived amino acid sequence of gru-1, with known sequences isolated from a suceptible population, identified 3 mutations that could be involved in BZ resistance. These results provide for the first time a structural basis for our hypothesis that in H. contortus BZ resistance is generated by a new mechanism: selection of specific functional β-tubulin isotype 1 genetic variants already present among isotype 1 variants in susceptible populations.

I 221 PROTEIN INTERACTIONS WITH A GENDER SPECIFIC GENE OF SCHISTOSOMA *mansoni*: CHARACTERIZATION BY DNase I FOOTPRINTING, BAND SHIFT AND UV CROSS-LINKING, Franklin D. Rumjanek, Ana L. Giannini and Simone Engelender, Dep.Biochemistry, ICB, Universidade Federal do Rio de Janeiro, Cidade Universitária 21910 Rio de Janeiro,Brazil

The Schistosoma mansoni gene F-10, encodes a major eggshell protein. Because this gene is expressed only in adult female worms, it was used as a probe to characterize DNA binding proteins which might have a role in the regulation of transcription. Restriction fragments of F-10 corresponding to different domains of the gene were obtained and tested against total and nuclear schistosome proteins, using the band-shift and DNase I footprinting methods. Sex-specific differences in footprints were only observed when nuclear proteins were tested. In this case the sites of interaction were detected in the restriction fragments corresponding to the 5' and 3' ends. Due to the occurrence of a steroid response element in the 3' untranslated end of F-10, UV-induced cross-linking between schistosome proteins and a synthetic oligonucleotide bearing the regulatory motif was carried out. The results revealed a major DNA binding protein with a molecular mass of 30 kDa, present in total protein extracts of both male and female worms. In contrast, only the male nuclear proteins were cross-linked to the oligonucleotide. The participation of steroid receptors in the regulation of F-10 transcription was further suggested by the detection of estrogen receptors through PCR amplification of schistosome cDNA. A model is proposed in which the activation of transcription of the F-10 gene may depend essentially on nuclear proteins, whereas cytoplasmic proteins might act as repressors.

I 222 NEUROPEPTIDE METABOLISM IN NEMATODES: PROPERTIES OF A MEMBRANE-BOUND AMINOPEPTIDASE AND ENDOPEPTIDASE, Mohammed Sajid and R.Elwyn Isaac, Department of Pure & Applied Biology, University of Leeds, Leeds, LS2 9JT, UK.

Cell-surface peptidases play a key role in the termination of the transmitter and hormonal actions of neuropeptides. With their active sites facing the extracellular space, these enzymes are well placed to hydrolyse peptides that approach the cell surface. In mammals, neuropeptide degrading enzymes display broad substrate specificities and are found in a range of tissues. Most invertebrate neuropeptides have an amidated C-terminus that prevents degradation by carboxypeptidases. Peptides blocked at both the N-and C- termini will also be resistant to aminopeptidases but will be susceptible to the action of endopeptidases.

We now report the presence of an endopeptidase that is firmly bound to membranes prepared from locomotory muscle of *Ascaris suum*. This enzyme can cleave peptide bonds on the amino side of phenylalanine; it is inhibited by phosphoramidon and thiorphan, both of which are inhibitors of mammalian endopeptidase 24:11. The endopeptidase activity has a neutral pH optimum but is only poorly inhibited by chelators of divalent metal ions. The same endopeptidase activity was also detected on the surface of isolated muscle cells.

Peptides such as AF1 (KNEFIRFamide) have multiple effects on locomtion in *Ascaris* suum. We have investigated the possible role of cell surface peptidases in the metabolism of neuropeptides by muscle cells. We show that the degradation of AF1 by muscle membranes is partly due to hydrolysis of the Glu-Phe bond by the phorphoramidon-sensitive endopeptidase. An amastatin-sensitive aminopeptidase also appears to be involved in the breakdown of this peptide. Some of the properties of the membrane-bound aminopeptidase will also be described.

I 223 A P-GLYCOPROTEIN GENE FAMILY FROM *HAEMONCHUS CONTORTUS*,

Nicholas C. Sangster, Department of Veterinary Pathology, University of Sydney, 2006, AUSTRALIA, Ronald D. Klein and Timothy G. Geary, The Upjohn Company, Kalamazoo, Michigan, 49007.

Overexpression of the P-glycoprotein confers multidrug resistance in cancer cells and *Plasmodium* by enhancing drug efflux. The function of the glycoprotein in normal tissues is unknown but it may be a cell volume regulated Cl^- channel. Three genes are expressed in gut cells of *Caenorhabditis elegans* and two are developmentally regulated. The genes of *H. contortus* were sought as part of an investigation into drug resistance.

Every P-glycoprotein gene contains two similar nucleotide binding sites which are conserved between species. Nested PCR reactions with degenerate primers coding for SGCGKST (sense) and DEATSALD followed by GQKQRIAI (antisense) were performed on cDNA libraries from adult worms and from eggs of *H. contortus*. Six distinct products from the adult library and 5 from eggs were cloned in *E. coli*. The fragments (263-345 b.p.) were sequenced. One group of fragments typified by #24 had 66% and 73% peptide homology or conservative substitution with equivalent fragments from mouse genes (amino and carboxy binding domains, respectively). One other adult fragment and 4 from egg had >96% peptide homology and >82% b.p. homology with #24. This probably reflects allelic differences between the individual worms used to create the libraries. The remaining 4 adult and one egg fragment showed considerable intra-specific variation with peptide homology of 62 - 79% to each of the other fragments. The results indicate that at least three genes occur in adults with a fourth in eggs.

I 224 STUDIES ON THE PROMOTER OF THE HSP70 GENE OF *Schistosoma mansoni*, Israel Schechter, Sylvia Neumann and Rivi Levy-Holtzman, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel

Schistosomes have a complex life cycle in which they are exposed to different environments and temperatures. Since heat-shock genes are activated in response to stress and during development, it is of interest to study the hsp70 gene family of schistosome. We have shown that hsp70 of *S. mansoni* reveal stage specific expression (miracidia+, sporocyst+, cercaria-, schistosomula+, adult worm+) and that hsp70 expression is regulated by a developmental program and by stress. We have isolated from *S. mansoni* a genomic clone containing the complete coding region of hsp70, and 5' flanking DNA containing typical promoter elements (TATA-box, CCAAT and HSE) at appropriate location from the transcription initiation site. Two heat shock responsive elements (HSE) that differ from the consensus sequence, CnnGAAnnTTCnnG, by one (HSEI) or three (HSEII) base changes were identified. Schistosome extracts with active transcription factors revealed by the gel retardation assay were prepared. Analyses of parasite extracts from different developmental stages showed that the presence of active heat shock transcription factor (HSTF) correlates with the pattern of hsp70 gene expression. Cercarial extracts did not show binding with ^{32}P-labeled HSEI or HSEII. Extracts of young schistosomula (4 hr after transformation) and of adult worms kept at 37°C or 42°C, showed binding of HSEI but not of HSEII. Young schistosomula showed highest levels of the (putative) active HSTF. The HSEI-HSTF interaction is specific because it is inhibited by cold HSEI and not by HSEII or other DNA fragments. Since the hsp70 promoters of *S. mansoni* and of other eukaryotes reveal similarities in the sequence and organization of the regulatory elements, we prepared constructs of the schistosome hsp70 promoter linked to the CAT reporter gene that were used to transfect CHO cells. The transfected cells showed heat shock dependent activation of CAT. These findings indicate that functional evaluation of schistosome promoters can be done (at least in part) in foster cell-lines of other eukaryotes.

I 225 DEVELOPMENTAL EXPRESSION OF GENES INVOLVED IN GLUCOSE UPTAKE AND METABOLISM IN *SCHISTOSOMA MANSONI*, Patrick J. Skelly, Charles B Shoemaker, Dept of Tropical Public Health, Harvard School of Public Health, Boston 02115.

We have cloned a set of cDNAs from *Schistosoma mansoni* which encode glucose transporter proteins (SGTPs) and proteins involved in glucose metabolism. Glycolytic enzyme cDNAs obtained include those encoding triose phosphate isomerase, glyceraldehyde 3 phosphate dehydrogenase and enolase. To permit examination of proteins involved in oxidative glucose metabolism, we used the polymerase chain reaction to clone fragments of two mitochondrial proteins - malate dehydrogenase (SMDH, a tricarboxylic acid enzyme) and subunit 1 of cytochrome oxidase (SCOX1, part of the electron transport complex). Other cDNAs used include hexokinase (SHEX), phosphogen kinase and actin. Following Northern Analysis and quantitation using the PhosphoImager from Molecular Dynamics we investigated the steady state transcript levels of all of these cDNAs in whole cercariae, cercarial tails, schistosomula and adult male and female parasites. Our most striking finding is that cercarial tails exhibit a large and co-ordinate level of expression of the genes involved in glycogen catabolism, ie all of the glycolytic and mitochondrial genes. Clearly the expression profile of tails is geared toward aerobic energy production. No GTP transcripts are detected and the SHEX level is low. Schistosomula express GTPs, SHEX as well as all glycolytic cDNAs but mitochondrial SMDH and SCOX1 expression is depressed relative to cercarial tails. This finding supports the notion that schistosomula metabolize glucose anaerobically and undertake little aerobic, oxidative metabolism which requires mitochondrial gene expression. Adult parasites are prodigious glucose consumers and both males and females express high levels of GTPs. Glycolytic transcript levels are relatively high in adults as, surprisingly, are levels of the mitochondrial transcripts. The latter finding suggests that adult parasites retain the capacity to generate at least part of their energy requirements through aerobic, oxidative metabolism.

I 226 A *CAENORHABDITIS ELEGANS* MEMBER OF THE NUCLEAR HORMONE RECEPTOR GENE FAMILY IS EXPRESSED DURING EARLY EMBRYOGENESIS, Ann E. Sluder and Gary Ruvkun, Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

As a means of identifying potential development control genes in *C. elegans* we probed genomic libraries with degenerate oligonucleotides encoding a highly conserved region within the *DNA binding* domain of nuclear hormone receptors. The predicted gene product of one clone isolated from this screen, *crf-2*, is 50% identical to the mouse thyroid hormone receptor within the DNA binding domain but exhibits no significant similarity to known receptors outside of this domain.

To obtain clues to the function of *crf-2* we have examined its expression pattern. *In situ* hybridization experiments (D. Greenstein, pers. comm.) and developmental Northern blot analysis detected *crf-2* transcript in early to midstage embryos and in gravid hermaphrodites. Analysis of mutants defective in aspects of germline development has shown that *crf-2* is transcribed in the maternal germline.

To define the *crf-2* expression pattern further, a *crf-2/lacZ* fusion gene has been constructed and used to transform worms. Immunostaining of transgenic animals detects fusion protein in particular embryonic cells from just before gastrulation through mid-embryogenesis. Zygotic expression of the fusion gene is sufficient to generate the observed pattern. Antibodies against *crf-2* protein have recently been obtained; immunostaining experiments are underway to compare the expression of the native protein with that of the fusion protein.

The physical map location of *crf-2* places it in a genetically well-defined region of Chromosome I. The *crf-2* gene does not appear to correspond to any of the existing mutations in the region, so we have initiated efforts to obtain a mutation in *crf-2*. Efforts to probe the *crf-2* mutant phenotype using antisense RNA are also underway.

I 227 ORGANIC ACID EXCRETION BY *ASCARIS SUUM* AND *HAEMONCHUS CONTORTUS*: IMPLICATIONS FOR MICROENVIRONMENTAL pH REGULATION AND DRUG ABSORPTION, David P. Thompson, Norman F. H. Ho, Sandra M. Sims, Craig L. Barsuhn, Eileen M. Thomas and Timothy G. Geary, Upjohn Laboratories, Kalamazoo, MI 49001

The *in vitro* excretion kinetics of organic acids and absorption kinetics of organic electrolytes by the gastrointestinal nematodes *Ascaris suum* and *Haemonchus contortus* were quantified to determine if metabolic endproducts generate and maintain a microenvironmental pH within the aqueous compartment of the cuticle. Both species excreted high levels of organic acid endproducts at relatively constant rates and in apparently fixed molar concentration ratios over the course of 24 h (*A. suum*) or 12 h (*H. contortus*) incubations. The predominant organic acids excreted by *A. suum* were 2-methylbutyric, 2-methylvaleric and acetic; in *H. contortus* they were acetic and propionic. The accumulation of organic acids in the incubation medium was associated with changes in the pH of the medium until a limiting, constant pH, in the vicinity of the pK_as of the acids was reached. Whether the initial pH of the medium was 7.5 (HEPES-buffered) or 3.25 (glycine-buffered), it eventually plateaued at ~5.0 (*A. suum*) or ~5.5 (*H. contortus*). The rates of organic acid excretion and absorption of benzoic acid, p-nitrophenol and aniline by both parasites were not affected by initial pH or buffer capacity of the medium, or by mechanical (*A. suum*) or ivermectin-induced (*H. contortus*) ligation of the nematode's intestine. The ligation procedures used reduce solute accumulation by the intestine of these nematodes by >95%. These results suggest that *A. suum* and *H. contortus* excrete organic acids across their cuticles, and that this process maintains the pH at the host-parasite interface at relatively constant levels. Since the microenvironmental pH of the cuticle controls the amount of uncharged acidic or basic permeant available for diffusion into the nematode, it has important implications for electrolyte absorption.

I 228 AEROBIC AND ANAEROBIC ENERGY METABOLISM DURING THE DEVELOPMENT OF HAEMONCHUS CONTORTUS.
A.G.M. Tielens, Laboratory of Veterinary Biochemistry, Utrecht University, Utrecht, The Netherlands.

In the various stages of their life cycle, parasites have to adapt to different environments; the availability of external food and oxygen varies widely. These variations in the external conditions influence the energy metabolism of parasitic helminths.

We investigated the energy metabolism of Haemonchus contortus, a parasitic nematode in sheep.

The free-living L3 stage larvae were found to degrade glucose mainly to CO_2 (via the Krebs cycle). Acetate and propionate were the main other end products of carbohydrate metabolism. Also in the early parasitic stage (XL3 = exsheathed L3) half of the glucose was metabolized via the Krebs cycle. In the adult, on the other hand, most of the degraded glucose was metabolized to propionate and acetate in an anaerobic ratio of 2 : 1. ^{13}C-NMR studies revealed some peculiarities in the anaerobic carbohydrate metabolism of Haemonchus.

Furthermore, the observed changes in metabolism show that, during their development, in these worms a transition occurs from succinate oxidation via succinate dehydrogenase (SDH) in the Krebs cycle, to the reversed reaction: reduction of fumarate to succinate. In Escherichia coli two different enzymes can be expressed for these reactions: SDH and also fumarate reductase (FRD). It is not known yet whether the same is true in parasitic helminths.

To investigate the nature of the enzyme(s) involved in the oxidation of succinate and the reduction of fumarate, the subunits are characterized. A degenerate PCR was used to clone and subsequently sequence subunits of SDH. Indications were found that two different SDH-B subunits exist, which have a different developmentally regulated expression.

I 229 NEMATODE ACETYLCHOLINESTERASES: SEVERAL GENES AND MOLECULAR FORMS OF THEIR PRODUCTS.
Jean-Pierre Toutant[1], Jean Bergé[2] and Martine Arpagaus[1,2]. Différenciation Cellulaire et Croissance, INRA, 34060 Montpellier (1) and Biologie des Invertébrés, INRA, 06606 Antibes (2), France.

Caenorhabditis elegans possesses three genes coding for three acetylcholinesterases (AChEs) with different catalytic properties. We have characterized the products of two genes (*ace 1* and *ace 2*) in *Steinernema carpocapsae*, a Rhabditidae close to *C. elegans* but presenting a higher AChE activity.

The two types of AChE were distinguished by their different sensitivity to eserine. *Ace 1* codes for a protein of approximately 90 kDa which is found under a major amphiphilic 14S form. This molecular form likely associates, by disulfide bonds, a tetramer of hydrophilic catalytic subunits to one non-catalytic (structural) hydrophobic component. *Ace 1* product is also found as hydrophilic 7S and 12S forms that likely correspond to hydrophilic dimers and tetramers of subunits. *Ace 2* codes for an amphiphilic catalytic subunit of about 65 kDa (4S) that associates into a membrane-bound dimeric form (G2a for amphiphilic) sedimenting at 7S. PI-PLC converts this G2a form into a hydrophilic G2 form (G2h) indicating that this type of catalytic subunit possesses a glycolipidic domain that mediates the membrane attachment. A complete scheme of molecular forms of *Steinernema* AChEs is presented (a). The two types of membrane association are similar to those previously identified:

-in amphiphilic G2 forms of vertebrate and *Drosophila* AChEs (glycolipid-anchored dimers, see b)

-in amphiphilic G4 forms of AChE in mammalian brains (hydrophobic structural component, see b).

C.elegans AChEs molecular forms were found similar to those in *Steinernema*. In *C. elegans* however we demonstrated directly the relationships of each gene with the corresponding molecular forms by an analysis of AChE in the mutants: *ace1*, *ace2*, *ace3* and *ace1/ace2*.

(a) Arpagaus et al., 1992, Eur. J. Biochem. 207, 1101-1108.
(b) Toutant and Massoulié, 1988, Hbook. Expl. Pharmacol. 86, 225-265.

I 230 GENETIC ANALYSIS OF MATING IN STRONGYLOIDES RATTI USING DNA FINGERPRINTING.

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Little work has been carried out on the genetics of parasitic nematodes. In *Strongyloides*, previous cytological studies have suggested that, following mating between free-living generation males and females, the male gamete penetrates the female gamete but that the sperm pronucleus then degenerates and the female gamete develops asexually. This process has been termed "pseudogamy". If true, it follows that the male has no genetic input into the next generation. The existence of free-living males who do not contribute genetic material to the next generation thus presents an evolutionary conundrum.

To test whether these earlier observations were valid, we have analysed the progeny of controlled crosses between genetically distinct clones of *S. ratti* in order to determine the source of the progeny's genetic material. Two clones of the parasite were produced by infecting rats with individual infective L3s. Each clone was shown to possess its own characteristic DNA "fingerprint", using as a probe a human multilocus minisatellite sequence denoted 33.15. In humans, the alleles that make up such fingerprints are known to be inherited in a Mendelian fashion.

Crosses were made between a virgin male of one clone and a virgin female of the other clone. The progeny were then cloned into rats and these clones analysed using the probe 33.15. In each clone bands characteristic of both the male and female parents were present. Approximately 50% of the bands in each clone appeared to be derived from the male, suggesting that mating was typical of higher eukaryotes. It can be concluded that pseudogamy does not occur in *S. ratti*.

I 231 *SCHISTOSOMA MANSONI*: CLONING OF A HOMOLOGUE TO THE MOUSE MYOGENIC TRANSCRIPTION FACTOR MYOD, David S. Weston, Rodrigo D. Cantu and W. Michael Kemp, Department of Biology, Texas A & M Universtiy, College Station, TX 77843

In *S. mansoni* muscle-specific gene expression is highly regulated throughout the lifeclcle of the organism. In adult stage parasites tropomyosin, myosin heavy chain, actin, and paramyosin are expressed at considerably higher levels as compared to cercaria and egg stages. Little is known as to how schistosomes regulate gene expression during these drastic developmental changes. However, it is known that in other organisms there exists a small family of myogenic transcription factors that play an important role in muscle-specific gene expression. Therefore, degenerate oligonucleotides directed toward the conserved basic-helix-loop-helix (B-HLH) domain of the mouse myogenic factor MyoD were designed. These primers were used in the polymerase chain reaction (PCR) on reverse transcribed adult *S. mansoni* poly(A) RNA. A predicted 140 bp product was obtained and directly cloned into the pCR2000 vector. The deduced translation of the sequenced PCR product yielded a single open reading frame which had 89.5% and 86.8% identity with the *Drosophila melanogaster* and *Musculus musculus* MyoD proteins, respectively. Other high scores included the *Xenopus lavis* myogenic factor (86.8%) and the *Rattus norvegicus* myogenin (73.7%). (Supported by the Office of Naval Research; NIH #AI-26505; Texas Advanced Research Program #010366-100)

I 232 PCR DETECTION OF *WUCHERERIA BANCROFTI* DNA IN SERUM, BLOOD AND MOSQUITOES, Steven A. Williams*+, Suzanne Chanteau# and Min Zhong+, *Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD 20892; #Institut Territorial de Recherches Medicales Louis Malarde, Papeete, Tahiti, Polynesie Francaise; +Clark Science Center, Smith College, Northampton, MA 01063.

Extensive screening of *Wuchereria bancrofti* genomic DNA libraries was conducted in an effort to identify repeat DNA sequences appropriate for PCR-based parasite detection assays. A family of dispersed repeats consisting of about 1000 copies of an AT-rich 200 base pair sequence was identified. Oligonucleotide primers were designed which hybridize to the conserved 5' and 3' ends of this repeat sequence. PCR with these primers and *Taq* polymerase was able to efficiently amplify as little as 0.1 pg of total genomic *W. bancrofti* DNA. When *W. bancrofti* DNA is present, a single band at 200 base pairs is observed. No bands are observed when human, mosquito, or *Brugia* DNA are tested in the PCR reaction. A rapid and simple method using silica for extacting DNA from lysed parasites in blood and mosquitoes was found to provide an ideal template for the PCR reaction. Following PCR, 10 ul of a 50 ul PCR reaction is run on an agarose gel. As few as one microfilaria in 50 ul of blood was efficiently detected using this method. The method was also successful at detecting one L3 larva mixed with 50 mosquito heads. Finally, when *W. bancrofti* DNA was added to 100 ul of human serum or 100 ul of whole blood, as little as 1 pg was efficiently detected. These results indicate that this simple PCR-based assay will prove to be useful in detecting *W. bancrofti* microfilariae in individuals with low titer infections. The assay will also prove useful in evaluating control programs in which large numbers of mosquitoes must be screened for the presence of *W. bancrofti*. Finally, this assay may prove useful in detecting circulating or "free" DNA in the blood or serum of infected individuals. A study is being conducted to test this hypothesis.

I 233 TISSUE DISTRIBUTION OF A C. ELEGANS ANTIGEN THAT REACTS WITH ANTI-$GABA_A$ RECEPTOR ANTISERUM. A. Wolstenholme, D.Laughton, P.Harris and G.Lunt. Dept. of Biochemistry, University of Bath, Bath BA2 7AY, UK.

The nematode nervous system is of interest as a model for more complex organisms, and also as a target for the development of novel agents against parasitic species. GABA is the major inhibitory transmitter at the neuromuscular junction, and pharmacological evidence suggests that the nematode GABA receptor is distinct from mammalian $GABA_A$ or $GABA_B$ receptors. Using a polyclonal antisera prepared against purified rat brain GABAA receptors (a kind gift from Dr. R. Olsen, UCSF) and fluorescence microscopy, we have detected specific staining in C. elegans. Specific reactivity with the antiserum was detected in glpharyngeal gland cells and in regions of the mid- and hind-gut. Cross sections indicated that the reactive antigen was located in the brush border and endotube of the gut.This is a somewhat surprising location for a GABA receptor, and may indicate antigenic cross-reactivity between the rat $GABA_A$ receptor and a component of the nematode gut.