Biology of Parasitism 2000

Marine Biological Laboratory

The Biology of Parasitism summer course is one of several run by the Marine Biological Laboratory, located at Wood's Hole, on Cape Cod, Massachusetts. This course is the longest, lasting just over 9 weeks, and comprises of four modules covering the cell biology, immunology, biochemistry and molecular biology of parasites in addition to a bioinformatics workshop week.

A day in the life of a BOP student

Each day began with seminars from invited speakers, all leading researchers who are currently at the forefront of parasitology. After introducing the topic, the speaker described ongoing work within their lab and outlined questions that still remain to be answered in that particular area of parasitology. There was then an opportunity to question the lecturer and meet informally for lunch before the class undertook a program of lab work which lasted the rest of the day (and sometimes even into the early hours of the morning!).

Cell Biology

BOP (as it is known to insiders) this year began with a 2 week module examining the invasion of the apicomplexan *Toxoplasma gondii*, an intracellular parasite which is able to invade virtually all cell types of the body. It is worldwide one of the most prevalent parasites and is normally harmless, but causes problems when it infects patients who are immunocompromised. This has become a problem of late with the onset of the AIDS

epidemic. We looked at several aspects of the invasion of this parasite under the guidance of David Russell (Cornell) and David Sibley (Washington University).

To invade host cells *Toxoplasma* must actively move and bind to the host cells which it will invade (Sibley, 1998). Gliding can be observed using fluorescently labelled antibodies to the parasite surface coat (figure 1). The first experiment was designed to look at the components of the cell biology of this parasite that are important in attachment and invasion. We used agents that affect cell signalling, intracellular calcium concentrations and the *Toxoplasma* cytoskeleton, incorporating the gliding assay to assess the effect. It was found that all of these components were required for binding to the host cell. However calcium and an unknown molecule which can be inhibited by the PKC inhibitor, calphostin C, were required for attachment. As expected, actin was needed for actual entry into cells (Dobrowolski, 1997). The role of the microneme proteins, which are released on contact with the host cell, was also examined. Inhibiting cell signalling using stausporine abrogated the secretions of these proteins.

Toxoplasma is known to enter cells via active invasion as opposed to host cell phagocytosis (Morisaki, 1995). The aim of the final experiment in this module was to set up a demonstration of active invasion and also to address the issue of how the parasites obtain the nutrients they require to survive and multiply once they have gained entry into the host cell. Using labelled tracer molecules and immunoelectron microscopy we were successful in examining the endocytic network of eukaryotic cells, active invasion and molecule trafficking to the parasitophorous vacuole.

Immunoparasitology

After a weekend break, and an end of module BBQ, the course moved onward to 2 weeks

of immunoparasitology. This module examined the immune response of the host to

Schistosoma mansoni, the causative agent of Bilharzia (Edward Pearce, Cornell), and to

the protozoan *Leishmania donovani* (Phil Scott, University of Pennsylvania).

If vaccines against parasitic diseases are to be developed, it is important to understand the

immune responses mounted by the host against these organisms. This module attempted

to examine the immune responses to *Leishmania* and *Schistosoma*. First if all we looked

at the role of host and parasite genetics in the ability of macrophages from different

mouse strains to kill different *Leishmania* species. This is an important area of research

with the current realisation that both host and parasite genetics can play a role in the

nature of the immune response mounted (Afonso and Scott, 1993; Scott and Farrell,

1998).

Cysteine proteinases have been implicated as virulence factors in *Leishmania*. The

observation in preliminary experiments that parasites deficient in cysteine proteinases

induce enhanced host immune responses suggest that somehow this phenomenon may be

due to the ability of these proteinases to modulate the immune response (Alexander *et al.*,

1998) With parasites devoid of cysteine proteinases constructed by Jeremy Mottram and

Graham Coombes (University of Glasgow) (Mottram *et al.*, 1996) we found no evidence

for cysteine proteinases acting as virulence factors at the level of macrophage killing.

Finally in this section we looked at the role of dendritic cells (DC) in promoting T helper

2 (Th2) responses to schistosome eggs. It is known that antigens located on the egg of

Schistosoma are potent inducers of Th2 responses (Vella and Pearce, 1992). However the

exact mechanism by which this occurs is unknown. The role of the DC in initiating a Th2 response on exposure to SEA's was examined. One possible mechanism may be by the production of IL4, a potent inducer of Th2 responses (O'Garra, 1998). We did not obtain clear results regarding the ability of DC's to produce IL-4. However we did observe that DC's exposed to SEA's seemed to express less of the surface marker CD40. This surface marker is necessary to produce IL12, a factor involved in triggering Th1 responses. Is it possible that SEA's trigger Th2 responses due to a failure to trigger Th1? Indeed this hypothesis was also suggested by *in vitro* analysis. DC's exposed to SEA's had a much reduced capacity to excrete IL-12.

Biochemical Parasitology

After a week of bioinformatics, and a 4th July parade, the class were launched into the biochemical parasitology under the auspices of Meg Phillips (University of Texas) and Murray Selkirk (Imperial). The class were divided into two groups looking at different systems. My group worked with Meg Phillips looking at *Trypanosoma brucei*, the causative agent of African sleeping sickness. Sleeping sickness affects up to 1 million people in Africa every year and currently there is no satisfactory chemotherapy against this parasite. The drugs that are available to treat *Trypanosoma brucei* infection are now becoming obsolete due to spreading resistance and new therapies are urgently needed.

Difluoromethyulornithine (DFMO) is used for treatment of this disease but is required in large doses to be effective (Kuzoe, 1993). In addition some isolates of the *rhodensiese* strain are naturally resistant (Bacchi *et al.*, 1993; Iten *et al.*, 1997). This agent is an inhibitor of ornithine decarboxylase, an enzyme catalysing the first step in the

biosynthesis of polyamines. The polyamine spermidine is incorporated into trypanothione, which is a molecule required for redox balance in this organism. One possible antitrypanocidal mechanism of action of DFMO may be the abrogation of trypanothione. In addition to spermidine, trypanothione is composed of another molecule known as glutathione. A study treating mice with buthionine sulphoxamine (BSO), an inhibitor of the glutathione pathway enzyme γ-glutathionyl cysteine synthetase (γGCS), indicated this enzyme as another possible chemotherapeutic target against sleeping sickness (Arrick et al., 1981).

The aim of this module was to generate some biochemical and structural information about these enzymes to provide information about the enzyme active site, which could be used to aid inhibitor design. This was accomplished by expressing recombinant enzymes with point mutations and carrying out kinetic analysis on these mutant proteins to ascertain which residues are important for their action. The crystal structure of ODC has been solved (Grishan et al., 1999) but crystals of γGCS have never been successfully made. Therefore the second part of this module involved investigating the conditions that are conducive to making γGCS crystals in order that they could later be X-rayed to determine the structure of this enzyme. This was successfully accomplished. The first crystals of GCS are shown in figure 2. Although not in an appropriate configuration to undertake X-ray crystallography, further crystallisation trials will now be undertaken in Texas to solve the structure of this enzyme.

Molecular Biology of Parasites

BOP2000 concluded with investigations into RNA metabolism in Trypanosomes (Christian Tschudi and Elisabetta Ulla, Yale) and transfection of the malaria parasite

Plasmodium berghei (Andy Waters, Leiden). Once again the class was split into two. I chose to study RNA metabolism.

The precise removal of intervening sequences for nuclear pre-mRNA's by cis-splicing is catalysed within complicated ribonucleoprotein complexes known as spliceosomes (Ares & Wiser, 1995). In the pre-mRNA's there are certain signals which indicate sites that have to be spliced. The sequence elements for cis-splicing are shown in figure 3. In higher eykaryotes spliceosome assembly is initiated by the binding of U1 snRNA on the spliceosome to the 5' splice site (5'SS). Thus U1 is required for cis-splicing in these organisms.

Until recently it has been thought that trypanosomes lack these sequences in their pre-mRNA's and therefore do not undergo cis-splicing. However Mair *et al.*, (2000) have shown that the poly (A) polymerase of some trypanosomes do indeed have an intervening sequence in their pre-mRNA's which must be cis-spliced in order for the mRNA to be translated to make this enzyme. It was ascertained during BOP 1999 that the U1 region of the spliceosome is also necessary for cis-splicing in Trypanosomes. The question we were trying to address was whether the U1 region in the snRNA of trypanosomes, as in the higher eukaryotes, interacts with the 5'SS of the pre-mRNA. BOP 1999 students were successful in engineering defects in the U1 region of the spliceosome and abrogating cis-splicing of poly (A) polymerase. This year we attempted to mutate the defects made last year to restore splicing.

In addition to attempting to look at the mechanism of action of the U1 region by restoring base pair mutations, we also investigated the regulation of the expression of this gene.

We were able to show by mutagenesis and subsequent plasmid expression that the D1 region (100 base pairs downstream of the gene), A box (110 base pairs down stream) B box (157 base pairs down stream) were positive regulators of the expression of this gene.

RNA interference (RNAi) is a recently described mechanism through which mRNA expression is down-regulated by gene-specific double-stranded RNA. The RNAi phenomenon has been described in several eukaryotic organisms, including *T.brucei*. The finding that RNAi is a widespread phenomenon implies it has an important function. As far as *C.elegans* is concerned one function of RNAi is thought to circumvent the effects of transposition in this nematode (Ketting *et al.*, 1999).

It has already been shown that transient expression of tubulin dsRNA from plasmid constructs or electroporation for synthetic tubulin dsRNA causes degradation of tubulin mRNA (Ngo *et al.*, 1998). Constructs used to elicit RNAi in a heritable and inducible fashion consist of sense and antisense sequences separated by a stuffer fragment sequence (figure 4). In undertaking RNAi in *T.brucei* there always appeared to be a residual amount of tubulin mRNA that was resistant to degradation. We were able to ascertain that the sequence of the stuffer fragment contributed to the efficiency of RNA degradation in this process.

The Benefits of Participating in the Biology of Parasitism Course.

I feel privileged to have had this opportunity to participate in the Biology of Parasitism 2000 Summer Course. It was very intensive and hard work. However in addition to having gained from studying parasitic systems other than my own, this course has given

me confidence in my laboratory work and an overview of the techniques that exist for studying parasites. I have met many researchers over the course of the summer in terms of other participants and invited speakers. All of them had a slightly different perspective on science and research which has helped to broaden my mind. No doubt I will collaborate with many of them in the future. I would highly recommend this course to other graduate students and post docs who undertake research in the field of parasitology.

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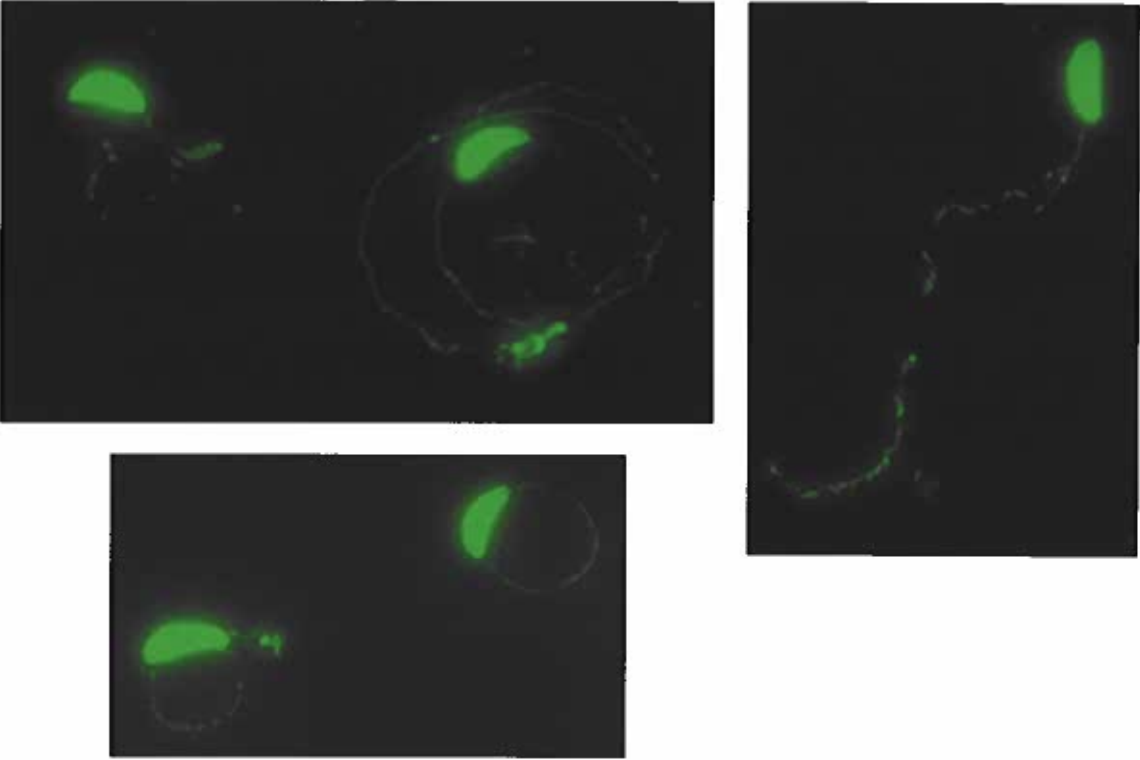
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Figure 1: The gliding of *Toxoplasma gondi*. Parasites were stained with FIT-C conjugated antibodies to surface antigen –1 (SAG1).
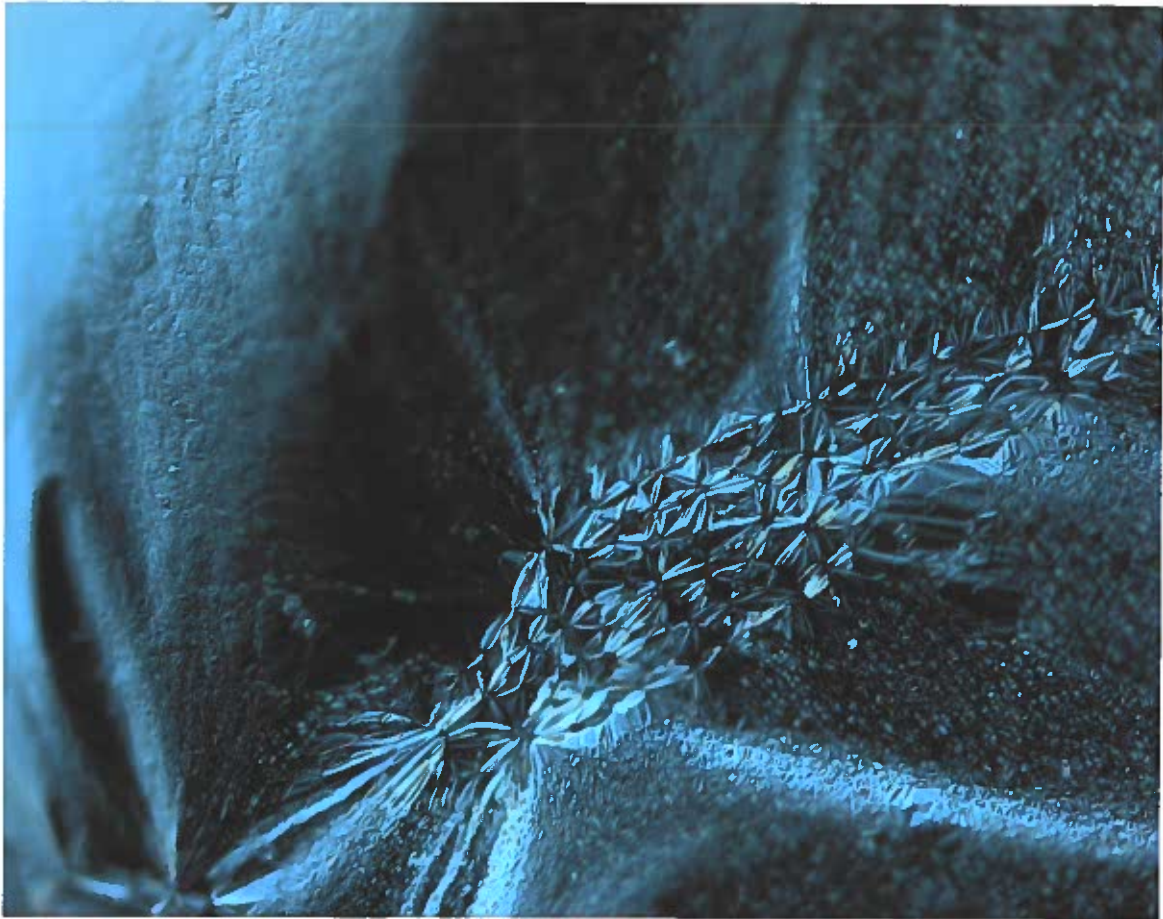
Figure 2: Crystals of *Trypanosoma brucei* γ-glutathionylcysteine synthetase.

5'SS   3'SS

BRANCHPOINT SEQUENCE

U1
SPLICEOSOME

POLYPYRIMIDINE
TRACT
20 - 40 NUCLEOTIDES

AG

EXONIC ELEMENTS

Figure 3: Features required for cis-splicing on pre mRNA's. In higher eukaryotes the binding of the spliceosome at the 5' spliceosomal site (5'SS) involves the U1 gene.

T7 PROMOTER   PROMOTER

PHLEO   SENSE   STUFFER   ANTISENSE

dsRNA

STUFFER

Figure 4: Schematic representation of the DNA construct used to elicit RNAi in *Trypanosoma brucei*. The construct contains the tetracycline regulatable PARP promoter (solid flag). The incorporation of the construct into *T.brucei* is verified using the phleomycin resistance gene (phleo) under the control of a T7 RNA polymerase promoter (open flag).