Cell Biology of Protozoan Parasites Addressed

7th Annual East African Regional Workshop Held

Last July, members of the ASCB International Affairs Committee traveled to Morogoro, Tanzania, to help offer a cell biology course—the first in a series. This course was a collaboration with faculty at the Genome Science Center, School of Veterinary Medicine, Sokoine Univerity of Agriculture (SUA), and the Seattle Biomedical Research Institute (SBRI). The course was co-hosted by Paul Gwakisa, SUA (below), and Patrick Duffy, SBRI (bottom of page). These scientists and their colleagues have run six previous workshops on malaria, so they are an experienced crew. Duffy will present a short description of some of this group's work at the ASCB Annual Meeting in San Francisco.

Course participants included SBRI cell biologists Michal Fried and ASCB member Marilyn Parsons, genomicist Peter Myler, statistician Bess Sorensen, and assistant Tony Getz. The ASCB sponsored Keith Gull, Eva Gluenz, and Bill Wickstead, Oxford University; Mahasin Osman, Cornell; David Roos, University of Pennsylvania; Dick McIntosh, University of Colorado; and Jonathan Adjimani, University of Ghana at Legon. Adjimani has volunteered to host next year's ASCB course. Also present was Janice Culpepper from the Bill and Melinda Gates Foundation and several additional SUA staff.

Co-host Paul Gwakisa, Sokoine University of Agriculture

Twenty-four students were selected from approximately 300 East African applicants on the basis of their training, experience, and prospects. Funding was provided by the Gates and Fogarty Foundation as well as the Carnegie Corporation of New York.

The course opened with lectures on the general cell biology of hosts and pathogens and unusual aspects of the "apicomplexan" parasite that causes malaria. This included a focus on their distinctive subcellular organelles and replicative mechanisms. Subsequent lectures covered many topics of interest to cell biologists, including the cytoskeleton and motility, cell-surface receptors, mechanisms of immune evasion, and iron metabolism. In each case, faculty compared properties of trypanosomes (which cause African sleeping sickness) and *Plasmodium* (which causes malaria). Additional lectures covered malaria during pregnancy and childhood and the development of a live vaccine now available for the cattle parasite *Theileria parva*. That parasite poses a major threat to livestock in East Africa.

Co-host Patrick Duffy (at left) talking to students and an American scientist

These lectures were interspersed with exercises of practical interest on subjects including tools for bioinformatic analysis of *Plasmodium* genomes (specifically focused on drug and vaccine target identification), discussions of drug and vaccine development, the basics of statistical analysis, and several journal club sessions.

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Workshop classroom in Tanzania

The discussions and journal clubs were an important feature of the whole course because they engaged the students and encouraged an environment of critical scientific thinking. Most of the students responded strongly and well to these sessions. This was especially noteworthy because many came from an environment where the culture of science teaching does not encourage questioning. The workshop faculty, therefore, placed a substantial emphasis on encouraging critical thinking and communication.

Session on Ethics

The workshop also included a session on ethics that was very well received. Duffy and Parsons role-played an unfortunate encounter between a young scientist with a valuable antibody and an aggressive senior scientist who wanted it. The show prompted a lively discussion about professional behavior. Participants then broke into small groups to discuss several case studies in scientific ethics. Students who had been somewhat quiet in other discussions seemed to feel more comfortable in contributing here.

Hands-on Exercises

Several afternoons were devoted to hands-on work by the students with either microscopic or FACS analysis of protozoan parasites. For microscopy, a lecture about imaging with light preceded brief instruction about the identification of different species of malaria parasites in Giemsa-stained blood smears. This was followed by a chance to try to make these discriminations. Students were then given protocols and reagents with which to stain blood smears that contained a high titer of *Trypanosoma brucei,* using either Giemsa or fluorescently labeled antibodies. Six bright field light microscopes were available for the former samples and one epifluorescence microscope for the latter samples. Immunostaining for variable surface antigens showed just how wily and distinct this parasite can be.

The FACS practical at the hospital also started with a lecture, in this case, on how a FACS operates and what it can do. Online resources were also available. Groups of two to three students each labeled *P. falciparum*-infected erythrocytes that had previously been fixed at various times after synchronization. They also labeled uninfected control erythrocytes, mammalian fibroblasts, and trypanosome parasites. The students were then able to run FACS analyses on these samples, following the replication of *Plasmodium* parasites and contrasting them with mammalian cells and trypanosomes. Students were also able to design and trouble-shoot various problems. They gained hands-on experience with differences in signal (e.g., DNA vs. light scattering), comparing *Plasmodium*, trypanosomes, human red blood cells, and mammalian fibroblasts.

Midway through the course, those who wanted to do so (all faculty and about half the students) traveled by car to the Mikumi Game Park. By starting before dawn, we were able to reach the park by 7:00 am, a good time to see animals as they began their day. We had nice sightings of giraffes (see below), water buffalos, hippos, crocodiles, elephants, wildebeests, a myriad of antelope, and countless beautiful birds.

Interactive Practicals

Week 2 began with 1.5 days of interactive practicals on the functional analysis of

Giraffes at Mikumi Game Park provided a mid-course diversion for course participants.

parasite genes, developed by Wickstead and Gluenz in the Gull lab. One exercise was based on a gene discovered by forward genetics in *Caenorhabditis elegans.* The gene is essential for intraflagellar transport in that organism. Students were asked to: (1) determine if *T. brucei* harbors a probable ortholog of this gene, using online resources, (2) apply Web-based tools to design an RNAi vector that would down-regulate this gene, and (3) think through how to analyze the resulting phenotypes. Data were available from work previously done in Oxford. As students designed their studies, results from real experiments were available for analysis. The data included growth curves, assessment of cell cycle stages in mutant vs. wild type populations, northerns, westerns, immunofluorescence, scanning and transmission electron micrographs, etc.

The second interactive practical was based on a screen of *T. brucei* cells transfected with an inducible RNAi library. This study involved selecting and characterizing the phenotype of a mutant defective in mitochondrial DNA segregation and identifying the gene responsible. Again, students had to start with an initial observation and discuss what data should be collected and why. Students received and discussed previously collected data and used it to plan the next step. Online analysis of the DNA sequence responsible for the mitochondrial phenotype revealed it to be a topoisomerase. Participants were able to conduct an entire RNAi phenotyping exercise using primary data, which again included northerns, westerns, growth curves, immunofluorescence, and transmission and scanning electron micrographs.

In both exercises, emphasis was placed on experimental design and data interpretation. This resulted in great student engagement. All of the groups also learned about professional and ethical computational processing of light microscopic images using NIH ImageJ.

There is great enthusiasm for the next ASCB-Carnegie African course offering, to occur in Ghana during the summer of 2009.

Planning Research

Following a tradition established in previous SUA workshops, each student worked with course faculty to plan and develop a research proposal, based on his or her B.Sc., M.Sc., or Ph.D. thesis, a planned grant application, or other personally important tasks. Proposals were grouped according to discipline, typically including about five students and two faculty. These groups met on the first day, and four times during the two-week workshops. These efforts culminated in a full-day event in which each student presented his/her proposal as a 10-minute PowerPoint presentation, followed by five minutes of questions.

Proposal development was complemented by sessions on planning applications, grant preparation, and assessment procedures, along with a discussion of funding opportunities for African scientists. These included international healthcare charities (WHO, Wellcome Trust, Gates, Carnegie, etc.) and U.S. and European government agencies.

Next Steps

Evaluations completed by the students suggested that this course was very well received, although discussions with students and among the faculty identified several possible improvements. There is great enthusiasm for the next ASCB–Carnegie African course offering, to occur in Ghana during the summer of 2009. This is an exciting opportunity to teach in a very different setting, passing on ideas, facts, methods, and general cell biological approaches in places where many of these ideas are new.

Thanks to Carnegie support, ASCB will be offering five more of these courses over the coming years. Volunteers to help make these events a valuable experience for all concerned would be very much appreciated! If you are interested, please write iac@ascb.org. ■

—Dick McIntosh and David Roos on behalf of the International Affairs Committee