Abstracts

Session 6
Molecular Biology

Plenary paper

S6.1 Pos genomic challenges: the assemble of *Plasmodium* signal transduction handling machinery

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Malaria afflicts millions of people and is still the major cause of death in the world. In the last few years the advances in genomic, expression profile as well transfection methodologies had provided great contribution to our understanding at the machinery level involved in *Plasmodium* cell physiology. Unraveling the mechanisms by which the parasite integrates signaling information resulting in cell function may be important for developing alternative strategies for therapeutic approaches to malaria. We have reported the role of a host hormone melatonin in *Plasmodium* cell cycle. Melatonin is known as a molecule that regulates the circadian rhythm of several vertebrates. By using confocal imaging we have identified a calcium and cAMP signaling cross-talk to transduce melatonin signaling by the parasite. We have also demonstrated that other triptophane-related molecules interfere with *Plasmodium* cycle inducing syncronization which might represent an evolutionary trick for the parasite to escape the host immune system. This data confirm our hypothesis that malaria parasites are able to sense the environment to modulate their own cell cycle. We are currently testing the function of four putative *Plasmodium* serpentine receptors by using heterologous system for transfection. Finally, we are also investigating for transcript level changes after *Plasmodium falciparum* parasites treated with the hormone melatonin. Parasites (18h after syncronization) were incubated for 12 hours with melatonin or control and RNA extracted. These samples were submited to real time analysis. Our data shows that the transcript levels changes after either the 6 or 12 hours incubation.

S6.2 Characterisation of the mitochondrial genome of *Plasmodium knowlesi* isolates from Malaysian Borneo

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Human infections with *Plasmodium knowlesi*, a malaria parasite typically found in long-tailed and pig-tailed macaque monkeys, are widely distributed throughout Malaysian Borneo. The mitochondrial genome of *P. knowlesi* was characterised to investigate the origins and demographic history of *P. knowlesi* parasites in this region. The mitochondrial genome of *P. knowlesi* (approximately 6 kb) was sequenced from 6 human isolates originating from different geographical areas within the Kapit division. For each isolate, the full length mitochondrial DNA of *P. knowlesi* was PCR amplified, cloned and sequenced. A battery of 28 forward and reverse walk-in primers were established for sequencing. Sequence alignment showed 5 unique haplotypes, which strongly support the hypothesis that human infections with *P. knowlesi* in the Kapit division were not due a recent clonal outbreak. The analysis also identified 10 single nucleotide polymorphisms (SNPs) across the genome with 8 of the SNPs occurring within the protein coding regions (*cox*I, *cox*III, *cyt*b). The mitochondrial genome of *P. knowlesi* in humans shows a high level of synonymous substitutions compared to non-synonymous substitutions within the protein coding regions, which probably suggests a long history of *P. knowlesi* parasites in Malaysian Borneo. Further characterisation of more mitochondrial genome haplotypes from both human and monkey hosts throughout Malaysian Borneo are essential to unveil the evolutionary history and the spread of *P. knowlesi* in the human population in this region.

S6.3 Molecular characterisation of *Plasmodium knowlesi* isolates from fatal and non-fatal human infections

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Plasmodium knowlesi, a parasite naturally found in pig-tailed and long-tailed macaques, was recently associated with human malaria in the Kapit Division of Sarawak, Malaysian Borneo. In addition, 4 malaria fatalities diagnosed as *P. malariae* by microscopy were recently reported in Sarawak. *P. malariae* is normally associated with uncomplicated disease and due to similarities between the blood stages of *P. knowlesi* and *P. malariae*, molecular characterisation of archival samples from fatal cases was conducted. DNA was extracted from thick blood films and analysed with genus- and species-specific nested PCR assays. PCR analysis showed that all 4 fatal cases had single *P. knowlesi* infections. In order to further confirm that these were *P. knowlesi* infections, the small subunit ribosomal RNA (SSU rRNA) and circumsporozoite protein (*csp*) genes from these samples were cloned and sequenced. Comparisons were made with other *P. knowlesi* sequences from 8 non-fatal human infections. DNA sequence analysis revealed a total of 7 genotypes for the asexually transcribed form of the SSU rRNA gene for the 12 isolates, with 2 genotypes from non-fatal cases being identical. Sequence analysis from the non-repeat region of the *csp* gene revealed 8 different genotypes. Two of the genotypes from non-fatal cases were identical to each other while another 2 shared a common genotype with 2 from fatal cases. These findings confirm that *P. knowlesi*, and not *P. malariae*, was the *Plasmodium* species for all the fatal cases and that there are no significant differences between *P. knowlesi* from fatal and non-fatal malaria cases.

S6.4 Characterisation of the circumsporozoite protein genes of *Plasmodium knowlesi* isolates from humans and monkeys

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Recent studies in the Kapit division of Sarawak showed that there was a large focus of naturally acquired human infections with *Plasmodium knowlesi,* a malaria parasite typically found in long-tailed (*Macaca fascicularis)* and pig-tailed (*M. nemestrina)* macaque monkeys. In order to determine whether human infections of *P. knowlesi* are zoonotic or acquired from other humans, the circumsporozoite protein (*csp*) genes of *P. knowlesi* were characterised from 15 human and 23 macaque monkey isolates in the Kapit Division. For each isolate, the *csp* genes were amplified, cloned and sequenced. Phylogenetic trees were constructed using the neighbour-joining method and DNA sequence data of the non-repeat region of the *csp* gene. Analysis of the phylogenetic trees showed that there is no separation in terms of clade formation between *P. knowlesi* isolates from monkey and human. Based on the non-repeat region of the *csp* gene, 19 genotypes were detected, 10 were isolates from monkeys, 7 from human infections and two genotypes were found in both monkeys and humans. These results demonstrate that monkeys are infected with *P. knowlesi* in the Kapit Division. Together with epidemiological and entomological data, these findings strongly suggest that macaque monkeys are the reservoir hosts of knowlesi malaria in the Kapit Division of Sarawak.

S6.5 Molecular characterisation of the *Plasmodium falciparum* chloroquine resistance transporter gene from different geographical locations

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Chloroquine resistance has been attributed to a single point mutation on the *P. falciparum* membrane transporter (*Pfcrt)* gene at amino acid position 76 (K76T). Further analysis of the *Pfcrt* locus identified 21 additional non-silent point mutations on the *Pfcrt* gene. Composite haplotypes constructed from the amino acids at each of these positions can be used to predict the origin of chloroquine resistance in particular isolates. In this study, 22-base *Pfcrt* haplotypes were constructed for 42 *P. falciparum* isolates from Jhapa, Nepal (n= 14), Binh Phuoc (n =10) and Dac Lac (n=10) in Vietnam and Lundu, Malaysian Borneo (n= 8). Seven different haplotypes were found in the 42 isolates in this study. Sixteen (38.1%) had a haplotype originating independently in South America and Papua New Guinea (PNG). This haplotype was found in 100 percent of isolates from Lundu and 8 (57.1%) of the isolates from Jhapa, Nepal. Eleven (26.2%) had a haplotype originating in Southeast Asia and they were found at both Vietnamese sites. Six (14.3%) of the isolates had a resistant haplotype formerly detected in Cambodia. Only 6 (14.3%) of the 42 isolates from the 3 different countries had *Pfcrt* haplotypes associated with chloroquine sensitivity. One of these isolates from Binh Phuoc had a novel motif within the sensitive haplotype. Two (4.8%) isolates had a novel haplotype and were only found in Nepal and one (2.4%) had a variant of the Southeast Asian haplotype. The origin and spread of chloroquine resistant *P. falciparum* at the different locations will be discussed.

S6.6 Arrested chromosome segregation at mitotic anaphase produces specific phenotypes in bloodstream and procyclic form *Trypanosoma brucei*

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Chromatid cohesin subunits are conserved among eukaryotes, and function to hold mitotic chromatids together throughout DNA synthesis until their segregation at anaphase. The separation of sister chromatids at the metaphase-anaphase transition is facilitated by the cleavage of one of the four cohesin subunits (Scc1), by a cysteine protease (separase, Esp1). We characterised the Scc1 orthologue in *Trypanosoma brucei* and studied its expression profile during the cell cycle in bloodstream and procyclic form cells. *Tb*SCC1 is associated with the chromosomes prior to DNA synthesis at late G1. As nuclear S-phase progresses, the relative expression level of *Tb*SCC1 increases, and this is maintained throughout G2 of the cell cycle. At anaphase, *Tb*SCC1degrades and is disassociated from the chromosomes. *Tb*SCC1 specific cleavage site mutants were cloned and used to investigate the checkpoint regulation of cytokinesis in response to an arrest in anaphase. In the absence of chromosome segregation, procyclic form cells continue to divide to produce one nucleated and one anucleate cell (zoid), suggesting an absence of a checkpoint control between karyokinesis and cytokinesis. In contrast, cytokinesis is blocked in bloodstream form cells unable to cleave *Tb*SCC1. However, the kinetoplast duplication is uninterrupted to produce cells with multiple kinetoplast and flagella. The cell body initiates cleavage at the anterior pole but cell duplication is arrested. The difference in checkpoint control between the two life cycle stages could result from a loss of regulatory mechanisms in procyclic forms, or a difference in the relative timings of anaphase and the onset of cytokinesis.

S6.7 Asymmetric cellular division is essential for the morphogenesis of *Trypanosoma brucei* epimastigotes in the *Glossina* vector

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The ontogeny of *Trypanosoma brucei* is characterised by a number of discrete developmental forms, specific for the establishment and progression of infection in the mammalian host and insect vector. These include the cryptic stages undergoing nuclear and kinetoplast repositioning and asymmetric cellular division in the *Glossina* fly. We characterised the cell division cycle of *T. brucei* in *Glossina morsitans morsitans* by using the mitotic sister chromatid cohesion subunit *Tb*SCC1 as a molecular marker, and a nucleic acid stain (Hoechst 33342) to visualize the nucleus and kinetoplast. The expression profile of *Tb*SCC1 in the proventricular long trypomastigotes and pre-division epimastigotes indicate that these cells are in late S or G2 phases of the cell cycle. The nucleus takes on a unique elongated form only observed in these cell stages. The onset of the asymmetric cellular division is characterised by the anterior-lateral repositioning of the kinetoplast, the pooling of cytoplasm towards the posterior pole of the cell, and degradation of *Tb*SCC1 from the chromatins. The nucleus and kinetoplast segregates almost concurrently at this stage. The whole process of asymmetric division can be completed in the proventriculus with the production of long and short epimastigotes. The long post-division epimastigotes degenerate and the nucleus fragments. The short post-division epimastigotes migrate to the salivary glands and reinitiate DNA synthesis. Asymmetric division in *T. brucei* is a necessary facet of its life cycle as this generates short epimastigotes that transform to infective metacyclics in the salivary glands.

S6.8 Phylogenetic relationships of the Asian *Coptotermes* as inferred from mitochondrial DNA COII gene (Isoptera: Rhinotermitidae)

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The subterranean termites *Coptotermes* species have spread to many parts of the world, especially to tropical and subtropical countries. It is an economically important termite genus that causes damages to timber, wooden structures and agricultural crops. Despite its importance, there have been numerous confusions of the taxonomy of *Coptotermes* spp. In this paper, we report our findings based on morphological and mtDNA studies on 8 species of *Coptotermes* from Asia and Australia. Partial sequence of the cytochrome oxidase subunit II (COII) gene was obtained from 15 populations of *C. formosanus,* 9 populations of *C. gestroi*, 4 populations of *C. vastator*, 2 populations of *C. curvinagthus,* 2 populations of *C. acinaciformis* and one population each of *C. kalshoveni, C. frenchi* and *C. lacteus*. We analyzed 680 base pair fragment of the COII gene using neighbour-joining and maximum parsimony methods. Both methods resulted in trees with highly similar topologies. Bootstrapping indicated support for five clades: Australian *Coptotermes, Formosanus* group, *Gestroi* group, *Curvinagthus* group and *Kalshoveni* group. The results suggested: (1) *C. acinaciformis, C. frenchi* and *C. lecteus* are closely related species; (2) *C. gestroi* and *C. vastator* are conspecific. Across the gene, only five nucleotide differences were detected between *C. vastator* and *C. gestroi.* Among the 13 populations of *C. gestroi/vastator*, the genetic diversity range between 0 and 0.8%. Molecular approach used in this study promise an accurate identification of the weakly-defined morphospecies in this genus, which is crucial for the study of these insects in the urban environment in Asia.