Meeting report

# Molecular approaches to malaria: on the way to integration

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A report on the Molecular Approaches to Malaria meeting, Lorne, Australia, 4-8 February 2004.

The Molecular Approaches to Malaria (MAM) 2004 meeting was the second gathering of researchers studying the molecular basis of malaria. Compared to the first meeting (MAM2000) four years ago, the organizers registered twice as many abstract submissions, featuring a large number of accomplishments as well as the development of novel approaches in studies of this important disease. The completion of the genome sequence of *Plasmodium falciparum*, the main causative agent of human malaria, is clearly the main achievement of the past few years and the sequence resource has been instrumental for much of the recent development in this field. This report focuses on some of the main themes of the presentations at the meeting, which - by incorporating new techniques - are rapidly changing the focus of research within the protozoan parasitology research community.

## *Plasmodium falciparum* genomics

Representing the malaria genome-sequencing consortium, Matthew Berriman (Wellcome Trust Sanger Institute, Hinxton, UK) summarized the sequencing projects that have been ongoing at the institute since the completion of the *P. falciparum* genome in October 2002. Currently, three- to five-fold coverage of the genome sequence is available for about six other *Plasmodium* species, which are parasites of species ranging from mouse to human. This sequence coverage allows assembly of the partial genomes using the completed *P. falciparum* genome as a template. Phylogenetic comparisons revealed that about 60% of the genes in each genome are shared among the *Plasmodium* species, and Berriman described these as the gene-set of "an average *Plasmodium* parasite". The species-specific genes were mainly found at the telomeres of most of the chromosomes and at breaks of synteny in intrachromosomal regions. The species-specific genes are mainly involved in unique, species-specific processes of the parasites and thus are potential targets for antimalarial strategies.

David Roos (University of Pennsylvania, Philadelphia, USA) introduced the updated version of the Plasmodium Genome Resource [http://www.PlasmoDB.org], which includes the complete *P. falciparum* genome as well as the partial genome sequences of additional plasmodial species. In addition, the database incorporates datasets from two genome-wide gene-expression analyses and two proteomic analyses of developmental processes of the complex *P. falciparum* life cycle. The Plasmodium Genome Resource is an extremely valuable online resource for researchers, and following the example of the *Saccharomyces cerevisiae* genome database, it helps to enhance the collaborative spirit of the malaria research community, and to attract a number of new researchers into this field.

Taking advantage of the genome sequence, Geoff McFadden (University of Melbourne, Australia) and co-workers were able to identify amino-acid sequence requirements for targeting proteins to the plasmodial apicoplast - the non-photosynthesizing chloroplast-like organelle essential for an apicomplexan parasite's growth. Initially, a putative transit peptide was identified in a set of 68 plasmodial gene products that share a high level of homology with known chloroplast proteins in plants. Experiments with green fluorescent protein (GFP) fusion constructs verified the transporting properties of this signal peptide. In the absence of any primary or secondary structure in common, a single, positively charged sequence element present in each transit peptide was found to be sufficient for apicoplast targeting. Approximately 500 nuclear-encoded plasmodial proteins were found to contain this peptide element, making them potential apicoplast proteins. On the basis of these predictions, McFadden and colleagues were able to construct the apicoplast metabolic map, which includes the pathways for biosynthesis of fatty acids, isoprene and heme. This unique

organelle presents a great potential target for novel antimalarial chemotherapy.

The emergence of genomic sequence for rodent-parasitic *Plasmodium* species has brought a new dimension to the application of rodent models to malaria research. The linkage-group selection technique was used to identify genes essential for interactions between the *Plasmodium* parasite and its host in a study presented by Richard Carter (University of Edinburgh, UK). This technique uses a large array of genomic markers to identify factors essential for parasite survival in cells derived by a conventional genetic cross under specific selection conditions. In their initial experiment, Carter and co-workers demonstrated the involvement of the *pcmsp1* gene product in strain-specific host immunity. Currently they are refining the linkage-group selection method to identify additional factors for drug resistance and parasite-host interaction.

## (Epi)genetics of malaria antigenic variation

The subtelomeric regions of *P. falciparum* chromosomes almost exclusively encode several gene families of plasmodial surface antigens, including *var, rifin,* and *stevor*. Switches in gene expression within the *var* gene family are believed to be responsible for antigenic variation and thus the high virulence of malaria parasites. Artur Scherf (Institut Pasteur, Paris, France) and co-workers found that the telomeric and subtelomeric regions of each chromosome are compartmentalized into transcriptionally silent, compact chromatin at the periphery of the nuclei. In addition, homologs of all of the essential subunits of the *S. cerevisiae* gene-silencing complex, Sir1-Sir4 and Ku, were found in the *P. falciparum* genome. Chromatin immunoprecipitation studies indicated that the silencing complex is predominantly associated with the telomeres and subtelomeric regions of the *Plasmodium* chromosomes, and probably extends into the *var* gene coding regions. Although the detailed mechanism of the transcriptional switches within antigenic gene families remains to be elucidated, the findings presented by Scherf indicate that this process has an epigenetic character.

To validate the transcriptional switches *in vivo*, Hans-Peter Beck (Swiss Tropical Institute, Basel, Switzerland) reported results from a survey of the pattern over time of full-length *var* transcripts in the human population of regions where malaria is endemic. By following semi-immune children with mild malaria over a period of 4 months, Beck demonstrated that *var* gene expression is highly dynamic, with a mean of 1.7 *var* transcripts per infecting strain. A single patient could be infected with up to 14 different strains simultaneously. In spite of the highly dynamic pattern of *var* gene expression, however, a small number of transcripts were retained or recurred for up 10 weeks. The recurrence of several antigenic determinants indicates a limit to the antigenic variation, possibly due to structural constraints, and it presents a key opportunity for vaccine development.

## Proteomics and structural biology

Matthew Bogyo (Stanford Medical School, USA) demonstrated the power of combinatorial chemistry to identify and characterize potential drug targets in *P. falciparum*. Using a combinatorial library of suicide inhibitors of cysteine proteases, his group performed the first chemical 'knock out' in *P. falciparum*. The complete inhibition *in vivo* of falcipain 1, one of the main cysteine proteases, resulted in the abolition of merozoite invasion of host erythrocytes. Bogyo's group is aiming to extend this approach to other classes of plasmodial proteases, with the aim of identifying new drug targets.

Structural genomics of *P. falciparum* has traditionally been hindered by the extremely low efficiency of heterologous systems for the expression of plasmodial proteins. Evelina Angov (Walter Reed Army Institute of Research, Silver Spring, USA) used the codon-harmonization technique to enhance the expression of plasmodial merozoite surface protein (MSP1) in *Escherichia coli*. This technique analyses discordance in codon usage between *P. falciparum* and *E. coli* along a given gene. The plasmodial gene is then resynthesized *in vitro*, replacing every *P. falciparum* codon synonymously with the equivalently used codon from *E. coli*. Using this technique the yield of MSP1 production was increased 1,000-fold.

Raymond Norton (Walter and Eliza Hall Institute of Medical Research, Parkville, Australia) reported that the three-dimensional structure of the ectodomain of AMA1, one of the main plasmodial surface molecules, contains large sections of highly unordered structure. The small regions of ordered structure occur mainly around the disulfide-stabilized domains. Phage-display experiments determined that the stabilized regions are the main targets of protective antibodies synthesized by the host. In addition, the ordered structural domains are mainly exposed to the surface and contain the majority of the sequence polymorphisms, which are selected by immune pressure. Norton noted that preserving the three-dimensional structure of the vaccine-candidate molecule might be a key issue in the development of an antimalarial vaccine.

A better understanding of three-dimensional protein structures will have a broad impact on *Plasmodium* research in the near future. A sample of this development was presented by Amit Sharma (International Centre for Genetic Engineering and Biotechnology, New Dehli, India), whose group solved the crystal structure of Pfg27, a major *P. falciparum* gametocyte protein. Pfg27, which does not share any significant sequence homology with any known protein, was found to be made up of two pseudo-dyad-related repeats. This

surprising structural duplication occurs without any homology between the amino-acid sequence of the repeats. In addition, the crystal structure revealed potential SH3 and RNA-binding domains, which were undetectable in the primary amino-acid sequence. This work indicates that an increasing number of structural studies will be instrumental in a further annotation of the genome, and for an understanding of the physiological processes of the *Plasmodium* parasite.

This report would not be complete without acknowledging the insightful contribution of Lawrence Bannister (King's College London, UK). More than 40 years of his research into the ultrastructure of the *Plasmodium* cell has broadened our understanding of the biological processes in this highly specialized cell. As he remarked, even in this era when most research focuses on genomic and proteomic aspects, the integration of multiple approaches is essential for a full understanding of the malaria parasite and the future development of antimalarial strategies. MAM2004 featured a wide array of topics, ranging from genetic and biochemical approaches to immunological studies, and represented a major step forward on the way to wide, integrative approaches to combat one of the most lethal diseases on the planet.

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