Gene Regulation

Introduction

Our laboratory joined the Department of Medical Parasitology and Infection Biology at the Swiss Tropical Institute in October 2006. We study gene regulation in the malaria parasite *Plasmodium falciparum.* The success of our research activities relies on a variety of molecular and cellular biology techniques, which we share and establish in close collaboration with the Molecular Parasitology and Molecular Epidemiology unit. We are currently concentrating our efforts on two main projects: (i) investigating the epigenetic regulation of the virulence gene family *var,* and (ii) compiling an inventory of the nuclear proteome to identify and characterise novel regulatory proteins.

We believe that our lack of understanding of many fundamental processes in *Plasmodium* biology is a direct result of the paucity of research conducted on gene regulation and nuclear function in this important parasite. A key observation that highlights the importance of transcriptional control during the *Plasmodium* life cycle is the parallel transcription of functionally related sets of genes during the 48-hour development of the parasite within human red blood cells. These cascades of gene transcription facilitate the timely expression of essential parasite-specific processes such as host cell remodelling, expression of virulence factors and parasite invasion. Furthermore, different sets of genes are transcribed at different developmental stages in both the human and mosquito hosts. However, the regulatory mechanisms and nuclear factors involved are completely unknown. Apart from the recent bioinformatic identification of a family of putative transcription factors in apicomplexan parasites (ApiAP2), gene-specific regulatory proteins have hardly been detected, and to date not a single one has been functionally described.

Another example of the essential role of transcriptional control in parasite biology is the regulation of the *P. falciparum var* gene family. Here, recent work has uncovered a complicated scenario of epigenetic control mechanisms involved in mutually exclusive *var* transcription, switching and transcriptional memory. To accomplish this, the parasite is equipped with a substantial set of eukaryotic chromatin modifiers, which are recruited to *var* gene promoters by specific DNA-protein interactions. However, which of these factors are involved and how they interact to control *var* gene regulation is still a mystery. A further important role has also been attributed to the spatial and functional organisation of the nucleus. As the most intensely studied system of transcriptional regulation in *P. falciparum, var* gene regulation provides us with a unique tool to identify and study multilevel regulatory processes.

By combining targeted investigation of the *var* gene family regulatory system with a global approach to investigating gene regulation and nuclear function, we hope to generate important findings about essential transcriptional control strategies in *P. falciparum.* The objective of the approach is to understand critical processes in the biology of the parasite, to develop novel tools for interfering with the expression and antigenic variation of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), and to open new pathways to intervention. Our main questions are the following: (i) Can we identify the regulatory strategy of mutually exclusive *var* gene transcription? (ii) Are these processes also involved in the regulation of other subtelomeric virulence gene families (iii) Can we exploit the temporal, structural and spatial organisation of the nucleus to understand gene regulation in *P. falciparum*? and (iv) Can we identify regulatory factors that are unique to *Plasmodium* and/or other apicomplexan parasites?

5.1 Epigenetic regulation of the *var* gene family

Red blood cells (RBCs) infected with *P. falciparum* display parasite-derived proteins on their surface. Among these, the major virulence factor PfEMP1, which is encoded by the *var* gene family, plays a central role in severe disease. Surface-exposed PfEMP1 mediates binding of iRBCs to host receptors on endothelial cells, leading to sequestration of erythrocyte aggregates in the microvasculatory system. Furthermore, PfEMP1 undergoes clonal antigenic variation that facilitates chronic infection. The basis for this variation is provided by a complex regulatory strategy that controls mutually exclusive transcription of the *var* gene family. Only a single *var* gene is transcribed per parasite, while the remaining family members remain transcriptionally silent. Using genetically modified parasites, we recently showed that *var* gene promoters harbour essential information for *var* gene silencing, activation and mutual exclusion (Voss et al., Nature 2006;439(7079):1004-8). To understand the processes involved in epigenetic regulation of the *var* gene family in detail, we are focusing on specific DNA-

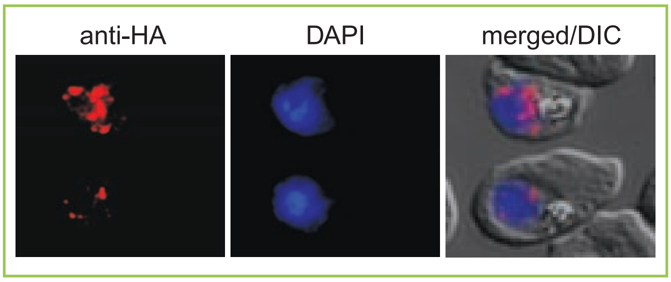

Figure 1: Localisation of the SPE2-binding protein in *P. falciparum* trophozoites at the periphery of the nucleus, visualised by immunofluorescence.

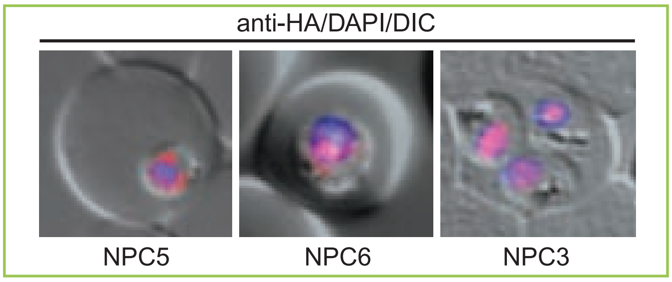

Figure 2: Immunofluorescence assays confirm the localisation of several novel protein candidates in the parasite nucleus.

The Gene Regulation team. (Photo S. Rusch)

protein interactions using regulatory DNA elements in *var* gene promoters as "bait" to identify interacting proteins.

We are particularly interested in dissecting the regulatory information contained in *var* gene promoters and 5′ untranslated regions (5′ UTRs). We would like to use this information to identify and characterise the regulatory proteins involved. To achieve this, we have developed a transfection-based reporter system using the dual reporter protein human dihydrofolate reductase (which confers resistance to the antifolate WR99210) fused to green fluorescent protein (hDHFR-GFP). This system allows us to monitor default *var* promoter activity by GFP fluorescence and real-time reverse transcriptase-polymerase chain reaction, and to select for promoter activation by WR99210 challenge. This approach has identified a region in the 5′ UTR that is essential for promoter activation as well as additional regions in the promoter which may play a role in silencing and mutually exclusive transcription. Experiments such as electromobility shift assays (EMSAs) and yeast one-hybrid screens are currently in progress to identify specific DNA-protein interactions involving these sequences.

The aim of a further project is to identify the protein(s) that bind to the well-described SPE2 regulatory sequence element located in subtelomeric *var* gene promoters. This interaction is highly specific and mediated by an unknown protein expressed exclusively in the schizont stage. We employed a large-scale affinity purification approach followed by mass spectrometry (Paul Jenoe, Biozentrum Uni Basel) to identify the SPE2 interacting protein as a member of the recently described ApiAP2 family of putative transcription factors. We confirmed the binding of this factor to SPE2 elements in super-shift EMSA experiments using recombinant protein expressed in both *Escherichia coli* and *P. falciparum.* Furthermore, immunofluorescence microscopy revealed a restricted localisation of a haemagglutinin-tagged version of this protein to discrete regions at the parasite's nuclear periphery (see Figure 1). This pattern is reminiscent of the distribution of chromosome-end clusters where subtelomeric *var* genes are naturally located. Indeed, chromatin immuno-precipitation expe-riments demonstrated that this protein binds to SPE2 elements *in vivo* (Henk Stunnenberg, University of Nijmegen). In other words, we have identified the first transcription factor of *var* genes, and work is now in progress to characterise the function of this protein. We believe the protein is essential for parasite survival, as several attempts to knock out the encoding gene have failed. This finding suggests that, besides regulating *var* gene transcription, the SPE2-binding protein may be involved in additional processes such as chromosome-end stability, nuclear structure and replication. Additional components that co-purified together with the SPE2-binding protein are also under investigation.

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5.2 Analysis of the *P. falciparum* nuclear proteome

A number of *Plasmodium* proteomic studies have already been published since the completion of various *Plasmodium* genome-sequencing projects. However, these studies mostly used whole-cell preparations, which makes it difficult to allocate hypothetical (i.e. novel) proteins to subcellular compartments such as the nucleus. To try and understand nuclear function in *P. falciparum* more broadly, and because we expect that a large number of proteins in the *Plasmodium* nuclear proteome will turn out to mediate parasite-specific regulatory processes, we are using biochemical fractionation of parasite nuclei followed by mass spectrometry-based protein identification. This procedure results in enrichment of nuclear proteins in different fractions reflecting, at least in part, the functional organisation of the nucleus. We have generated these protein fractions from ring-stage parasites, trophozoites and schizonts. The samples were analysed by mass spectrometry in the laboratory of Paul Jenoe (Biozentrum). The complex datasets, which contain a total of over 1,000 protein identifications, are currently being screened to eliminate false-positive hits. We will then combine bioinformatic analyses (Stuart Ralph, Univ. of Melbourne) and experimental approaches to validate our datasets and to define an inventory of the parasite's nuclear proteome. The nuclear localisation of several candidate proteins has already been confirmed using transgenic *P. falciparum* parasite lines expressing tagged versions of these proteins (see Figure 2). The results demonstrate the feasibility of our approach and open up a path towards a better understanding of nuclear function and regulatory processes in this parasite.

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