Cornelia Spycher Awardee 2006

Plasmodium falciparum causes the worst form of human malaria and is responsible for 1-2 million deaths annually. A vaccine is not available and resistance to drugs is widespread. One approach to tackle these problems is to identify new intervention targets. However, this is hampered by a limited understanding of many aspects of P. falciparum biology.
The morbidity and mortality associated with malaria is due to the asexual erythrocyte stages of P. falciparum. While most intracellular pathogens interact with an active host cell, the malaria parasite develops within the red blood cell (RBC) that is devoid of all organelles and any protein trafficking machinery. The parasite resides in a parasitophorous vacuole (PV), which is encircled by a parasitophorous vacuolar membrane (PVM). The parasite modifies its host cell by establishing membranous structures in the RBC cytoplasm. These comprise discshaped structures at the RBC periphery called Maurer's clefts (MC) with an elusive function.
In addition the parasite modifies the surface of the infected RBC by exporting own proteins that contribute to the virulence of P. falciparum. A key protein in this process is the variant surface antigen, P. falciparum erythrocyte membrane protein 1 (PfEMP1), which mediates adherence of infected RBCs to host endothelial cells. This is thought to prevent the infected RBC of being cleared by the spleen. Furthermore the parasite evades the immune system by a process called antigenic variation, the switching of the expression between different members of PfEMP1.
As mature RBCs lack a cellular trafficking machinery, the parasite establishes its own secretory system for exporting proteins beyond the parasite membrane (PM) through the PVM and across the host cell cytoplasm to the MCs and the RBC membrane. The mechanisms and molecular apparatus involved in this process are not completely understood. A classical signal sequence is sufficient to direct proteins into the endoplasmatic reticulum (ER) with default release into the PV. Onward transport across the PVM requires an additional signature termed Plasmodium export element (PEXEL) or vacuolar targeting signal (VTS).
The aim of this thesis was to characterize a novel transmembrane (TM) protein termed membrane-associated histidine-rich protein 1 (MAHRP1). It is transcribed exclusively in early stages, has a C-terminus with approximately 30% histidines present as DHGH repeats and localizes to the Maurer's clefts. MAHRP1 has no classical signal sequence and no PEXEL/VTS motif and it is thus unclear how it is directed to the MCs.
Histidine-rich proteins have been shown to be effective cation binders. The histidine-rich region of MAHRP1 was recombinantly expressed and used for interaction studies with the toxic waste product ferriprotoporphyrine (FP), which accumulates upon degradation of hemoglobin -a main nutrient source of the parasite. MAHRP1 specifically interacts with FP and binding stoichiometry correlates with the amount of DHGH repeats. The bound FP has increased peroxidase-like activity and is 10-fold more susceptible to H_2O_2-induced degradation compared with unbound FP. These properties of MAHRP1 suggest it may play a protective role against oxidative stress at the MCs.
To investigate the amino acids responsible for correct trafficking of MAHRP1 to the MCs, plasmids were generated encoding different green fluorescent protein (GFP)-tagged domains of MAHRP1 and parasites were subsequently transfected with these plasmids. Analysis of transfectants showed that the full length MAHRP1- GFP is successfully trafficked to the MCs, whereas the domains TM or TM-C-terminus were retained at the ER. Dissection of the Nterminus revealed a segment of 18 amino acids containing a motif with limited similarity to the PEXEL/VTS motif, and which is needed for export of MAHRP1 to the MCs.
Fluorescence photobleaching and time-lapse imaging techniques indicate that MAHRP1-GFP is initially trafficked to isolated subdomains in the PV/PVM that appear to represent nascent
MCs. The data suggest that the MCs bud from the PVM and diffuse within the RBC

cytoplasm before taking up residence at the cell periphery.
To understand the function of MAHRP1, a mutant with a mahrp1 disruption and MAHRP1 ablation was generated. MCs are still formed in the absence of MAHRP1 but the export of PfEMP1 is interrupted at the PM/PVM interface. As a consequence, no PfEMP1 is detected on the surface of infected RBCs. By contrast, export of other selected proteins appears to be uninhibited. This indicates that MAHRP1 plays an essential role in the export of major virulence factor PfEMP1 and thus represents an interesting intervention target in the battle against malaria.