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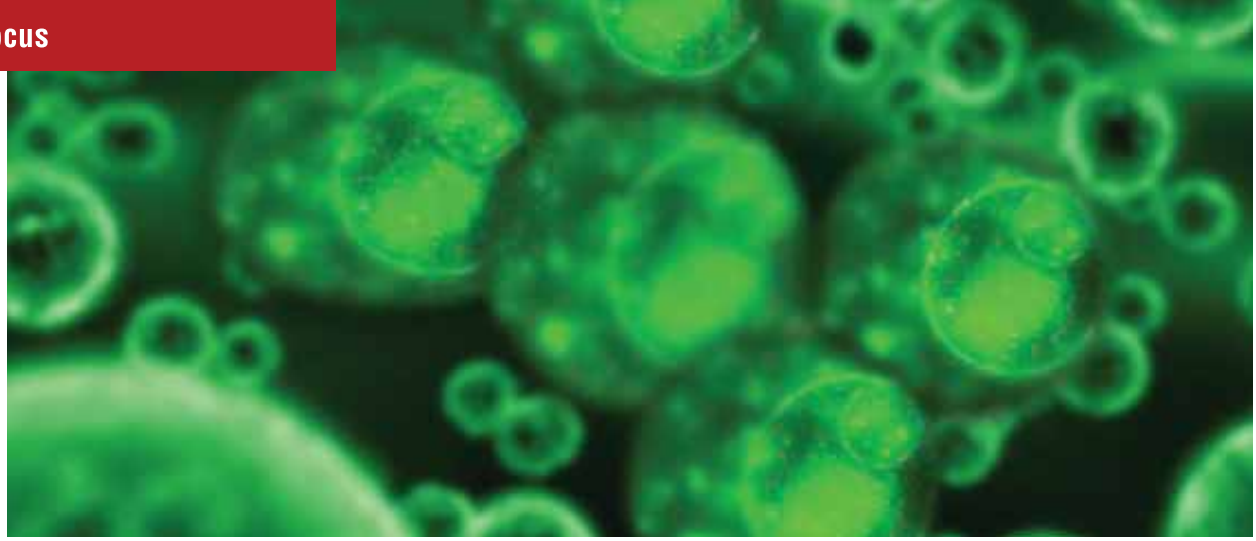

By Nicola Wittekindt

Identifying New Targets

For Antimalaria Drugs And Vaccines

Each year hundreds of millions of people are infected with *Plasmodium falciparum* malaria, suffering from various symptoms such as high fevers, headaches and anemia that in severe cases can progress to coma and death.

Even after more than a century of malaria research, all attempts to develop an effective vaccine against *P. falciparum* have failed due to the versatility of the parasite. Repeated emergence of resistance to antimalaria drugs, as seen lately for the very effective drug Artemisinin, threatens the few available means to battle the disease.

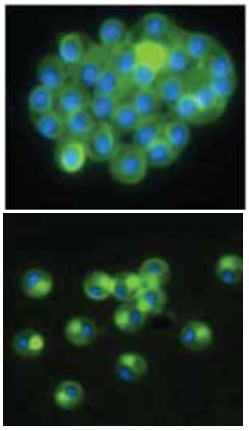

Fig 1. Localisation of potential protein targets in malaria parasites: Green Fluorescent Protein (GFP)-tagged proteins of the CIR multigene family (related to the STEVOR multigene family) were localised to membranes of (**a**) mature malaria parasites and (**b**) merozoites. Parasite nuclei are stained blue.

The development of effective new drugs and vaccines is the goal of Prof Peter Preiser from NTU's School of Biological Sciences. He aims at elucidating the biomolecular processes and mechanisms involved in the interaction between *P. falciparum* and its human host in order to detect molecular markers on the parasite that could serve as targets for the development of vaccines and anti-malaria drugs.

"We want to understand, why we are not able to develop a vaccine against the malaria parasite, and what exactly is the capacity of the parasites that enables them to get around the host immune response," says Prof Preiser.

He is looking specifically at the stage of the parasite's life cycle in which it infects the red blood cells – the stage that causes the actual disease.

After entering the human body through the bite of an infected Anopheles mosquito, *P. falciparum* – in its sporozoite life cycle stage – infects liver cells where it differentiates into merozoites. The merozoites escape into the blood and infect red blood cells, starting repeated cycles of multiplication, release from disrupted cells, and invasion of new red blood cells.

"The one time the parasite is outside of the cell is during the invasion process, so at this time it is potentially a target for immune attack or any kind of intervention. However, the parasite has developed a highly sophisticated mechanism of ensuring that it can get into the red blood cell," explains Prof Preiser.

Recognition of the red blood cells and the initial steps of their invasion are mediated by members of several multigene families encoding multiple closely related protein variants. Switching between these protein variants enables the parasite to evade recognition and eradication by the immune response (see Fig 1). Moreover, after successful invasion of a red blood cell, *P. falciparum* extensively modifies the surface of the host cell with unique proteins also encoded by multigene families. Certain protein combinations expressed on the surface of the red blood cell seem to be correlated to the severity of the disease.

"It's this complexity of protein modifications on the surface and how that links to virulence that we are trying to get a better understanding on," says Prof Preiser.

To shed light onto these mechanisms, Prof Preiser's team investigates the role of the reticulocyte binding protein homologue (RH) family in different stages of the invasion process, using *Plasmodium* lab cultures as well as mouse model systems infected with mice-specific *Plasmodium* strains. Studies of the parasite's behaviour in the mouse – where it can be manipulated and challenged by immunisation or by drug administration – gives valuable clues on parasite-host interaction.

Furthermore, severe malaria and high parasite burden were found to be correlated to the presence of rosettes – clumps of infected and uninfected red blood cells – in the patients' blood.

Prof Preiser's research team found that members of the STEVOR protein family (the abbreviation stands for sub-telomeric variable open reading frames) are expressed not only on the surfaces of the merozoite (Fig 1) but also on parasitised red blood cells. Their research also indicated that the STEVOR proteins are key players in attracting uninfected red blood cells to form rosettes with infected cells (Fig 2).

Rosette-formation of uninfected and parasitised red blood cells enables newly released malaria parasites to immediately invade fresh red blood cells in their vicinity. This strategy accelerates parasite propagation and minimises the time the parasites spend outside of cells, thereby largely preventing detection by the host immune system.

Besides the RH and STEVOR protein families, other so far unknown proteins play key roles in the invasion process and parasite virulence. Fifty percent of *Plasmodium's* more than 5,000 genes are unique to this genus and highly conserved among *Plasmodium* strains. The hypothetical proteins encoded by these unique genes presumably have key functions in *Plasmodium* and thus are highly interesting candidates for drug and vaccine development. Moreover, drugs targeted at these *Plasmodium*-specific proteins will most likely not result in side effects in human patients.

To get insights into possible functions of these unique genes, Prof Preiser has joined forces with Assoc Prof Zbynek Bozdech (also from NTU's School of Biological Sciences) on an extensive endeavour: The scientists attempt to profile the transcriptomes – the entire sets of genes expressed at any one time – of many different samples of *P. falciparum* using microarray technology.

Prof Bozdech had established earlier that gene transcription in *P. falciparum's* blood stage life cycle behaves like a developmental cycle. Every gene is only used once, leading to a cascade of regulation.

"These transcriptional studies can give a lot of insights on what genes are being used and at what point in time, and potentially allow to identify certain drug targets," explains Prof Preiser.

The transcriptional cascade can be influenced by treating the parasite with certain drugs or inhibitors in lab studies. In addition, parasite samples taken from patients that exhibit characteristic disease manifestations, certain blood cell abnormalities, or treatment failure and drug resistance, can be transcriptionally profiled.

Comparison of these data sets enables the researchers to identify transcriptional patterns and in particular to pinpoint changes to these patterns that provide clues to the functions of certain genes and proteins.

"If you have enough data you can start developing these interaction networks, which then give you a very powerful tool to predict function," says Prof Preiser. "When you look at a network and you have one protein with a known function in a specific pathway, you can assume that the other proteins in the network have functions in the same pathway."

The identification of genes – or transcriptional patterns – that are linked to severe disease or severe outcome, is one key goal of Prof Preiser's research. "We might be able to use these patterns as a predictive diagnostic tool for newly infected patients, or for identifying the real target in terms of intervention for preventing severe disease from developing. So there are lots of avenues that information would be valuable for," Prof Preiser adds.

Using cell biological methods, the researchers already succeeded in localising certain target proteins in the parasite, thus validating some of the predicted networks. Functional transcriptional profiling appears to be a very promising approach towards identification of potential targets for the development of new antimalaria drugs and vaccines.

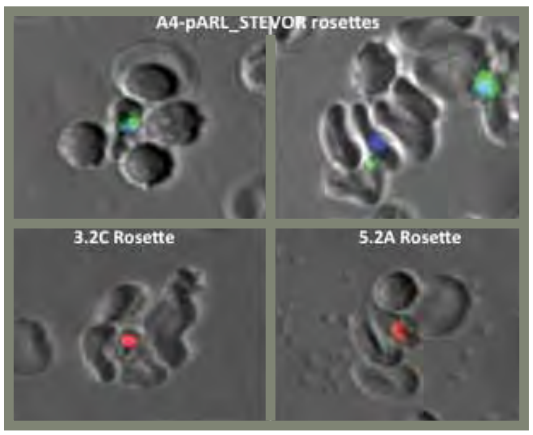

Fig 2. Rosetting: (**a**) Microscopic visualisation of rosettes that formed after Green Fluorescent Protein (GFP)-tagged STEVOR proteins were expressed in parasites. GFP-tagged protein expression is shown as green dots associated with the parasite. Parasite nuclei are stained blue. (**b**) Microscopic visualisation of rosettes. Parasite nuclei are stained red.