

Preliminary Investigation of Molecular Trafficking in Malarial Infected Red Cells

C. Magowan¹ and W. Meyer-Ilse²,

¹Life Sciences Division, Ernest Orlando Lawrence Berkeley National Laboratory,
University of California, Berkeley, California 94720, USA

and ²Center for X-Ray Optics, Ernest Orlando Lawrence Berkeley National Laboratory,
University of California, Berkeley, California 94720, USA

Investigation of the interactions between the intraerythrocytic malarial parasite *P. falciparum* and the host red blood cell has been the major focus of our biological x-ray effort at LBNL. We published the first extensive study of the development of parasites within normal human erythrocytes, and of the effects on parasite structural integrity of abnormal erythrocyte membranes and protease inhibitors, a class of potential anti-malarials (Magowan 1997). In that study we described newly elaborated carbon dense tubular and sheet-like structures that appeared to surround the parasite and extend into the red cell cytosol. We hypothesized these structures were components of the tubovesicular network (TVN), a network that is thought to be involved in protein and lipid transport. In a more recent study we immunolabeled molecules for x-ray microscopy with silver-enhanced gold-bead conjugated antibodies, demonstrating the success of this technique on both red blood cells and melanoma cells. This technique has enabled us to study the interactions between a parasite induced ligand on the surface of infected erythrocytes, and receptors on endothelial cells which mediate adhesive changes associated with the often fatal syndrome of cerebral malaria (Yeung 1998).

The malaria parasite is a eukaryotic cell that has adapted to a unique environment in which it is surrounded by the red cell plasma membrane and cytosol. The parasite itself has a plasma membrane, and sits within another membrane-bound compartment, the parasitophorous vacuole, enclosed by the parasitophorous vacuolar membrane (PVM). The PVM in turn extends into a series of complex, multicompartimented tubulovacuolar structures in the red cell cytosol known as the tubovesicular network (TVN). Sphingolipid synthesis in the infected red cell has been localized to the TVN which may be analogous to the *cis*-Golgi (Elmendorf and Haldar 1993; Elmendorf and Haldar 1994). While some data strongly suggest that protein transport in infected red cells can proceed via a classical Golgi secretory pathway (Hinterberg et al., 1994) or a functional but reduced Golgi (Ward 1997), other workers hypothesize that a morphologically and biochemically distinct "classical" Golgi complex may not exist (Banting et al., 1995).

Much remains to be learned about the nature of the pathways for trafficking molecules between the parasite and the external milieu. The mechanisms by which parasites transport molecules across the several membrane layers that surround them are of interest because these unique mechanisms may be vulnerable to inhibitors, they may provide information about previously unrecognized eukaryotic transport pathways, and they may provide a means of introducing chemotherapeutic agents directly to the parasite. The physical nature of routes for transport of nutrients, macromolecules and non-metabolized solutes has been debated. There may be a transient or stable parasitophorous duct of 50-70nm diameter which provides direct access between the intraerythrocytic parasite and the external milieu. Its existence was first suggested by the results of a study that used small highly fluorescent

latex spheres to investigate transport of macromolecules (Pouvelle et al., 1991). These results have been contradicted by other studies (Hibbs 1997; Fujioka and Aikawa 1993). A subsequent study hypothesized two distinct macromolecular transport pathways in malaria infected red cells (Goodyer 1997). Transmission EM in that study showed areas of apparent membrane continuity between the erythrocyte membrane and the parasitophorous vacuolar membrane which may constitute the "metabolic window" hypothesized by Elford *et al* to occur at contact sites or regions of close apposition between the host erythrocyte membrane and the PVM (Elford et al., 1995).

The studies currently underway at Beamline 6.1 extend our investigations of subcellular structures and processes that can be imaged using immunogold labeling, and ultimately, the tilt-stage capabilities of the x-ray microscope, to the study of protein trafficking in malarial infected erythrocytes. In the past, transport of proteins to and from parasites has been investigated, for most part, through models based on morphological analyses using fluorescence microscopy. With higher resolution x-ray microscopy and tilt-stage and tomographic reconstruction technology, we will utilize and extend the many tools and techniques that have been developed and tested in fluorescence analyses of malarial protein transport.

To these ends, we obtained two antibodies that are reported to react with the PVM and confirmed that they localize by indirect immunofluorescence with the intracellular parasite. We have acquired the expertise to reproducibly lyse red cells and release parasites using dipeptide induced selective osmotic shock (Elford and Ferguson 1993). We have devised additional steps to overcome the clumping of extruded parasites we encountered. Currently, stereo images can only be collected from dried samples which are manually tilted to gather images from two angles. Difficulties arose when we imaged dried samples of extruded parasites, in that we were unable to obtain the image clarity needed to see the parasites. The implementation of a cryo-tilt stage would eliminate these difficulties. Experiments are continuing to determine whether the extruded parasites can be imaged when they are labeled with antibody-conjugated gold beads enhanced with silver.

This research is supported by NIH grant #DK32094-10 and the United States Department of Energy contract #DE-AC 03-76SF00098.

References

1. Banting, G., Banting, J. and Lingelbach, K. (1995). A minimalist view of the secretory pathway in *Plasmodium falciparum*. *Trends in Cell Biology* 5, 340-343.
2. Elford, B. C., Cowan, G. M. and Ferguson, D. (1995). Parasite-regulated membrane transport processes and metabolic control in malaria-infected erythrocytes [Review]. *Biochemical Journal* 308, 361-374.
3. Elford, B. C. and Ferguson, D. (1993). Secretory processes in *Plasmodium*. *Parasitol Today* 9, 80-81.
4. Elmendorf, H. G. and Haldar, K. (1993). Identification and localization of ERD2 in the malaria parasite *Plasmodium falciparum*: Separation from sites of sphingomyelin synthesis and implications for organization of the Golgi. *Embo J* 12, 4763-4773.

5. Elmendorf, H. G. and Haldar, K. (1994). *Plasmodium falciparum* exports the Golgi marker sphingomyelin synthase into a tubovesicular network in the cytoplasm of mature erythrocytes. *J Cell Biol* 124, 449-462.
6. Fujioka, H. and Aikawa, M. (1993). Morphological changes of clefts in Plasmodium-infected erythrocytes under adverse conditions. *Exp Parasitol* 76, 302-307.
7. Hibbs, A., Stenzel, DJ, and Saul, A (1997). Macromolecular transport in malaria--does the duct exist? *Eur J Cell Biol* 72, 182-188.
8. Hinterberg, K., Scherf, A., Gysin, J., Toyoshima, T., Aikawa, M., Mazie, J. C., Dasilva, L. P. and Mattei, D. (1994). Plasmodium falciparum: the pf332 antigen is secreted from the parasite by a brefeldin a-dependent pathway and is translocated to the erythrocyte membrane via the maurer's clefts. *Exp Parasitol* 79, 279-291.
9. Magowan, C., J.T. Brown, J. Liang, J. Heck, R.L. Coppel, N. Mohandas, and W. Meyer-Ilse. (1997). Intracellular structures of normal and aberrant *Plasmodium falciparum* malaria parasites imaged by soft x-ray microscopy. *Proc. Natl. Acad. Sci. USA* 94, 6222-6227.
10. Pouvelle, B., Spiegel, R., Hsiao, L., Howard, R. J., Morris, R. L., Thomas, A. P. and Taraschi, T. F. (1991). Direct access to serum macromolecules by intraerythrocytic malaria parasites. *Nature* 353, 73-75.
11. Ward, G., Tilney, LG and Langsley, G. (1997). Rab GTPases and the unusual secretory pathway of Plasmodium. *Parasitology Today* 13, 57-62.
12. Yeung, J., Brown, JT, Nair, A, Mohandas, N, Meyer-Ilse, W. and Magowan, C. (1998). X-ray microscopic visualization of specific labeling of adhesive molecule CD36 and cytoadherence by *Plasmodium falciparum* infected erythrocytes. *Res Commun Mol Pathol Pharmacol.* 99: 245-258.

Principal investigator: Cathie Magowan, Life Sciences Division, Ernest Orlando Lawrence Berkeley National Laboratory. Email: CCMagowan@lbl.gov. Telephone: 510-486-6439.