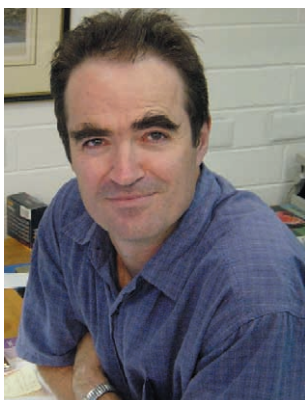


## Ion channels in the 'malaria-infected' red blood cell

Kieran Kirk explains how recent electrophysiological studies have provided new insights into the mechanisms by which the malaria parasite brings about a dramatic increase in the permeability of the red blood cell membrane to ions and nutrients

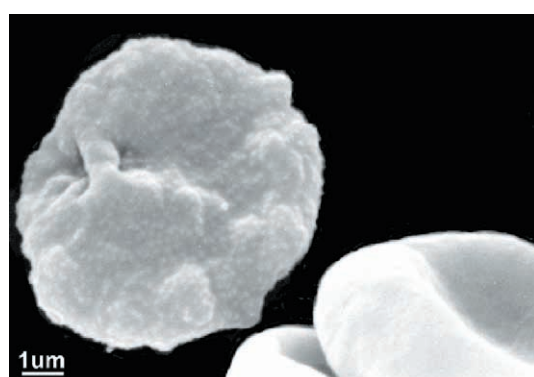
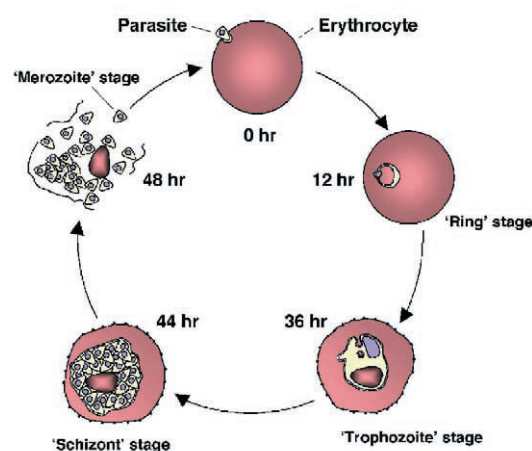


Kieran Kirk

Some hours after the invasion of a red blood cell by the single-celled malaria parasite (Fig. 1) there are profound changes in the physiological properties of the red cell membrane. A host of small molecules have been shown to enter infected cells more rapidly than they do normal, uninfected erythrocytes and this has been attributed to the presence in the infected cell membrane of so-called 'new permeability pathways'. These pathways are believed to serve a number of physiological roles, including the influx into the infected

cell of nutrients required by the parasite, and the efflux of potentially hazardous waste products derived from the parasite's active metabolism. The nature of these new pathways is not well understood, and their identity remains unknown. Over the last three years, however, electrophysiological studies from a number of different groups have provided new insights into the mechanisms underlying the altered membrane physiology of the parasitised red cell.

Early studies of the altered permeability of erythrocytes infected with the malaria parasite entailed the use of either radiolabelled transport substrates or measurements of the rate of haemolysis of infected cells suspended in isosmotic solutions of the substrate of interest. From these types of experiments it was evident that the pathways induced by the parasite accommodate a very wide range of low molecular weight solutes, including amino acids, sugars, nucleosides, vitamins and both inorganic and organic ions. Quantitative comparisons of influx rates showed the pathways to have a marked preference for anions over cations. Transport was shown to be non-saturable, and to be inhibited (with similar potency for a range of different transport substrates) by a range of classical 'anion transport inhibitors', including compounds such as furosemide, niflumic acid, glibenclamide and 5-nitro-2-(3-phenylpropylamino)-benzoic acid (or NPPB as it is better known). Together, the available transport data are consistent with much, if not all, of the increased flux of solutes into parasitised erythrocytes being via anion-selective (but nevertheless cation-permeable) channels of a single type.



**Figure 1.** Schematic representation of the (~48 hour) red blood cell stage of the life cycle of the human malaria parasite, *Plasmodium falciparum*, and scanning electronmicrograph showing a mature 'trophozoite-stage' infected erythrocyte together with two uninfected erythrocytes. The scanning electronmicrograph was provided by Professor D.J.P. Ferguson, University of Oxford

Ion-selective channels are best studied using electrophysiological techniques. Human erythrocytes are not the easiest cells to study electrophysiologically; their small size, and their ability to squeeze through narrow openings (and to thereby disappear up the barrel of a glass microelectrode), make them difficult targets. Nevertheless, several groups have recently been successful in making electrophysiological recordings of both uninfected and malaria-infected human erythrocytes.

The first detailed characterisation of the electrophysiological characteristics of infected human erythrocyte came from Desai and colleagues, who reported that in cells infected with mature parasites the whole-cell current is 150-fold larger than that of uninfected erythrocytes (Desai *et al.* 2000). The increased current was attributed to the activity of a small conductance ( $< 10$  pS) anion channel. The channel was inwardly rectifying (i.e. it passed current into the cell more readily than it did out of the cell), it was present at an estimated 1000 copies per cell, and it showed complex gating behaviour. The ion selectivity and pharmacological properties of the whole-cell currents showed close similarities to those reported previously on the basis of radiotracer flux and haemolysis experiments for the new permeability pathways induced by the parasite. Using a mathematical model to obtain quantitative permeability estimates from haemolysis experiments, the same group has shown recently that for several solutes there is good quantitative agreement between the permeabilities estimated on the basis of haemolysis experiments, radiotracer flux measurements and whole-cell current recordings (Wagner *et al.*, 2003), consistent with the channel underlying the inwardly-rectifying current being wholly responsible for the increased transport of at least some solutes into the infected cell.

Somewhat different results were

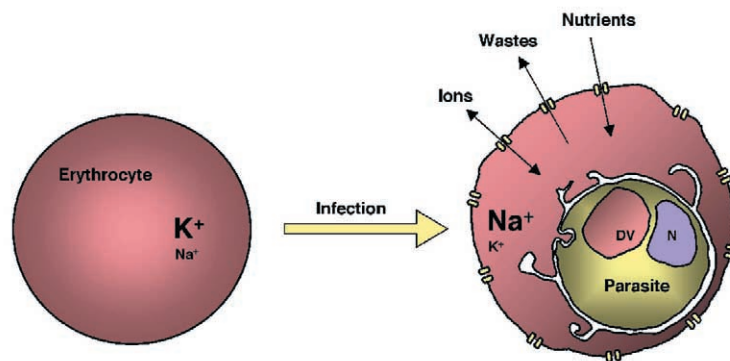
obtained in a study by Huber *et al.* (2002a) who, in whole-cell recordings of infected human erythrocytes, identified *two* discrete anion conductances, differing from one another both in their inhibitor-sensitivity and in their voltage-dependence. One was outwardly rectifying and the other inwardly rectifying. The conductances were diminished on treatment of the parasitised cells with reducing agents, and the same manoeuvre was shown to slow the rate of haemolysis of parasitised cells suspended in an isosmotic solution of the polyol sorbitol. In the same study it was shown that similar anion conductances could be induced in uninfected erythrocytes by exposing them to oxidizing agents, and that oxidative stress also induced haemolysis of uninfected cells suspended in an isosmotic sorbitol solution. On the basis of these observations it was postulated that the new permeability pathways induced in infected cells are endogenous erythrocyte channels, activated in response to the oxidative stress to which the host cell is subjected by the intracellular parasite. Of the two anion conductances characterized it was actually the outwardly rectifying conductance that had a pharmacological profile closest to that of the parasite-induced permeability characterised previously. A preliminary report of a differential effect of different polyols on the

outwardly rectifying conductance in infected cells (Huber *et al.* 2002b) is also consistent with the hypothesis that the channels underlying this conductance are permeable to small organic solutes of the sort known to enter the infected cell via the parasite-induced pathways.

The same group has reported the presence in uninfected human erythrocytes of an oxidation-induced cation conductance (Duranton *et al.* 2002) and have presented preliminary evidence that this conductance is activated in *P. falciparum*-infected cells (Tanneur *et al.* 2002). The conductance shows the same cation selectivity as has been reported for the transport of monovalent inorganic cations via parasite-induced pathways (i.e.

$\text{Cs}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+$ ; Staines *et al.* 2001), as well as showing an anion-dependence reminiscent of the anion-dependence of the uptake of both organic and inorganic cations into parasitised cells (e.g. Staines *et al.* 2001).

In another recent paper (Egée *et al.* 2002) a third group has obtained patch-clamp recordings of both uninfected and infected erythrocytes and have presented evidence for the activity in infected cells of an endogenous anion-selective channel, with a low linear conductance ( $\sim 15$  pS) and having properties similar, though not identical, to those



**Figure 2.** Schematic illustration of the physiological changes induced by the malaria parasite in its host red blood cell. The mechanism by which the intracellular parasite activates and/or inserts channels in the host cell membrane, and the identity of the channels themselves, are still to be resolved

of the parasite-induced channel originally described by Desai *et al.* (2000). Channels with the same properties could be activated in uninfected human erythrocytes either by the combination of protein kinase A and ATP, or by membrane stretch, raising the possibility that either one of these mechanisms might be involved in the activation of the channels in infected cells. In a small proportion (<5%) of excised inside-out patch-clamp experiments on uninfected cells a second anion channel, showing outward rectification, was observed. But whereas Huber *et al.* (2002a) observed an outwardly rectifying current in a majority of infected erythrocytes and have postulated that the enhanced permeability of infected cells to small organic solutes is attributable to the channels underlying this current, Egée *et al.* report that the outwardly rectifying channel was “never observed in infected cell patches”. They, like Desai *et al.* (2000), attributed the increased conductance of the parasitised erythrocyte membrane to a single channel type.

In summary, it is clear from the spate of recent electrophysiological studies of erythrocytes infected with the malaria parasite that the membrane of the parasitized erythrocyte has a much higher electrical conductance than that of uninfected erythrocytes. The identity and number of channel-types underlying this increased conductance is less clear. There is some evidence that the channel activity observed in infected erythrocytes is attributable to the activation of endogenous, normally quiescent, erythrocyte channels and a

number of different mechanisms of channel activation have been proposed (oxidative stress, membrane stretch, protein phosphorylation). The channel originally characterized by Desai *et al.* (2000), and showing a close (though not exact) resemblance to that described by Egée *et al.* (2002) does share many characteristics with the pathways responsible for the increased permeability uptake by parasitized erythrocytes of a wide range of low molecular weight solutes. This is consistent with, though not proof of, this channel underlying the increased permeability of the infected cell. However, the relationship between this channel and the multiple conductances reported by Huber and colleagues to be active in the membrane of parasitized erythrocytes (Huber *et al.* 2002a; Tanneur *et al.* 2002) is yet to be clarified.

Whatever the electrophysiological characteristics and molecular identity of the pathways responsible for the increased transport rates in the parasitized red blood cell, there is growing evidence of the significance of these pathways for the intracellular parasite and its host cell (Fig. 2). At least one essential nutrient required by the parasite (the water soluble vitamin pantothenic acid) has been shown to be reliant on these pathways to gain entry into the cell (Saliba *et al.* 1998). Recent studies using mathematical models developed by Lew and colleagues have revealed that the leakage of Na<sup>+</sup> and K<sup>+</sup> (down their respective gradients) via the parasite-induced pathways, is responsible for the conversion of the erythrocyte cytosol

from a high K<sup>+</sup>/low Na<sup>+</sup> medium to a high Na<sup>+</sup>/low K<sup>+</sup> environment for the intracellular parasite (Staines *et al.* 2001; Lew *et al.* 2003). Observations such as these highlight potentially important roles for the parasite-induced pathways in the infected cell and underscore ongoing interest in the possibility that these pathways might be suitable targets for new and much-needed antimalarial drugs.

### Kieran Kirk

Australian National University  
School of Biochemistry and Molecular Biology

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## Staff update!

### Casey Early

Casey joined the Society's London office in February 2003 to take over from David Sewell as the accountant. He is a qualified Chartered Accountant and had previously worked with the firms' auditors, haysmacintyre, for four years. Casey studied at Loughborough University and obtained a BSc in Maths with Economics, followed by an MSc in Financial Economics. Casey's interests include travelling, films and weight training.

### Sai Pathmanathan

Sai joined the Society in January 2003 as the new Education Officer and is taking on schools' liaison and some of the external relations work. Sai completed her BSc (Hons) in Biological Sciences at Queen Mary and Westfield, University of London and had just finished a DPhil in Neurosciences at the Department of Biochemistry in Oxford before joining the Society. Sai's interests include music, arts and crafts and voluntary work with youth and homeless.