

## CIC-2 Channels in Erythrocytes

Ekaterina Shumilina<sup>1</sup> and Stephan M. Huber<sup>\*2</sup>

Departments of <sup>1</sup>Physiology and <sup>2</sup>Radiation Oncology, University of Tübingen, Germany

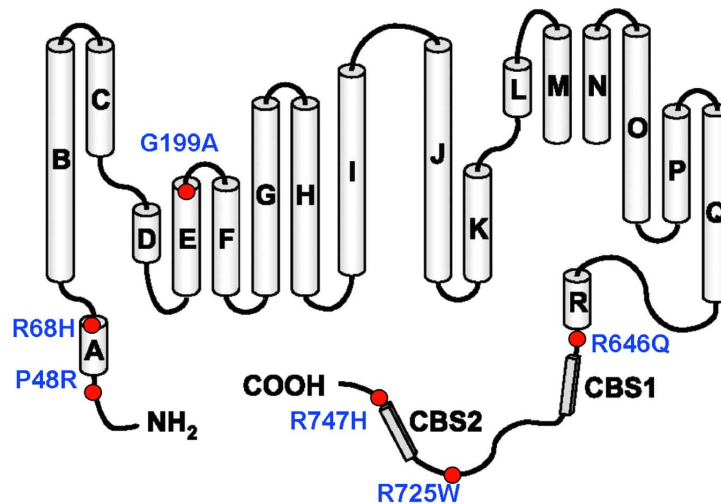
**Abstract:** CIC-2 is a ubiquitously expressed plasma membrane Cl<sup>-</sup> channel that reportedly controls the ionic environment in mouse retina and testis. Beyond that, CIC-2 might sense cellular energy status and cellular stress by its carboxy-terminal cystathionine-beta-synthase (CBS) domains and by its molecular interaction with the heat shock protein Hsp90, respectively. In mature human and mouse erythrocytes, CIC-2 is activated by oxidative stress and by malaria infection. This article describes possible function of erythrocyte CIC-2 channels for the programmed death of oxidatively injured erythrocytes and for the regulatory volume decrease of malaria-infected erythrocytes.

**Keywords:** Patch-clamp whole cell recording, red blood cell, chloride channel, oxidative stress, cystathionine-beta-synthase domain.

### THE CLC-2 CHLORIDE CHANNEL, A SENSOR OF CELLULAR STRESSES

The human *CLCN2* gene encoding the inwardly rectifying chloride channel CIC-2 is located on chromosome 3q26. It comprises 24 exons which were first sequenced by Thiemann *et al.* in 1992 [1,2]. The recently identified crystal structure of a bacterial CIC orthologue defines the molecular structure consisting of 18  $\alpha$ -helices that exhibit a complex

upon hyperpolarization. Moreover, CIC-2 channels are reversibly activated by hypotonicity-induced cell swelling [7,9] and lowering of the extracellular pH [8-10]. In addition, CIC-2 activity depends on the intracellular Cl<sup>-</sup> concentration [11]. CIC-2 mRNA has been detected in every tissue and cell line examined [1]. Accordingly, hyperpolarization-activated CIC-2-like Cl<sup>-</sup> currents have been observed in various tissues and cells.



**Fig. (1).** Model of the CIC protein (modified from [27]) showing the positions of polymorphisms analyzed in the laboratory of the authors.

topology and form a homodimer interacting at a broad interface [3] (Fig. 1). Each subunit forms its own pore (protopore) and selectivity filter. Several splice variants of CIC-2 have been described [2,4-6] showing altered channel function [2].

The halide selectivity sequence of CIC-2 is  $\text{Cl} \geq \text{Br} \geq \text{I}$  [1]. In *Xenopus* oocytes [1, 7, 8] as well as in transfected mammalian or insect cells [9] CIC-2 currents activate slowly

The cytosolic C-terminus of CIC-2 channels comprises two cystathionine-beta-synthase (CBS) domain. CBS domains (Fig. 1) are evolutionarily conserved and usually come in tandem repeats which form an intramolecular dimeric structure (CBS pair). The truncation of CBS-2 probably disrupts CIC channel function as demonstrated for CIC-1 [12]. CBS domains can bind adenosine-containing ligands [13] and the CBS domains of CIC-2 have been demonstrated to bind AMP, ATP, or S-adenosyl methionine [14]. Replacement of ATP by AMP reportedly accelerates the opening and closing kinetics of CIC-2 channels [15]. This might suggest that the CBS domains of CIC-2 function as sensors of cellular energy status.

\*Address correspondence to this author at the Department of Radiation Oncology, University of Tübingen, Hoppe-Seyler-Str. 3, 72076 Tübingen, Germany; Tel: +49-(0)7071-29-82183; Fax: +49-(0)7071-29-4944; E-mail: stephan.huber@uni-tuebingen.de

The heat shock protein Hsp90 interacts with CIC-2. This interaction results in greater channel activity due to increased cell surface channel expression, facilitation of channel opening, and enhanced channel sensitivity to intracellular Cl<sup>-</sup> concentration. The association of Hsp-90 with CIC-2 may enable CIC-2 to sense cellular stress such as elevated temperature, ischemia, or oxidative reagents [16]. Similarly, oxidative stress stimulates surface expression of the intracellular anion channel CIC-3 [17].

## CLC-2 FUNCTIONS IN INTERCELLULAR COMMUNICATION

The physiological function of the CIC-2 chloride channel in its native system has been discussed intensively [18]. CIC-2 is broadly expressed in plasma membrane. The strong dependence of CIC-2 gating on cell volume and its relatively sensitive volume set-point implicates that CIC-2 might be involved in the regulatory volume decrease (RVD) of eukaryotic cells after cell swelling [1,7,8]. However, except for malaria-infected erythrocytes [19] (see below), this hypothesis was not supported by experimental data obtained from knock-out models.

According to its localization to apical membranes of epithelial cells CIC-2 might contribute to transepithelial Cl<sup>-</sup> and fluid transport and to gastric acidification [20]. CIC-2 has also been detected in fetal lung and collecting duct and a function of CIC-2 in branching morphogenesis of these organs has been suggested [5, 21, 22]. Moreover, localization of CIC-2 in rat brain close to inhibitory synapses suggests a CIC-2 function in GABAergic transmission [23]. Seizure sensitive gerbils exhibit an enhanced CIC-2 expression in the hippocampus probably compensatory for a reduced GABAergic postsynaptic inhibition [24]. Loss of CIC-2 might result in neuronal hyperexcitability. A susceptibility locus for common idiopathic generalized epilepsy has been mapped to human chromosome 3q26 [25] which is close to the human *CLCN2* gene [26]. However, a report [27] that three different heterozygous mutations in the human *CLCN2* are associated with idiopathic generalized epilepsy has been retracted recently [28], calling a role of CIC-2 in the pathogenesis of epilepsy into question [29-31]. Accordingly, the established CIC-2 knockout mouse model (*clcn2*<sup>-/-</sup> mice) do not suffer from spontaneous seizures [18]. Except blindness and a leukoencephalopathy that progresses with age, *clcn2*<sup>-/-</sup> mice exhibit only mild neurological deficits [31]. In contrast to the proposed CIC-2 function in human epithelia, *clcn2*<sup>-/-</sup> mice do not show any defect in lung or kidney development and display normal gastric acidification.

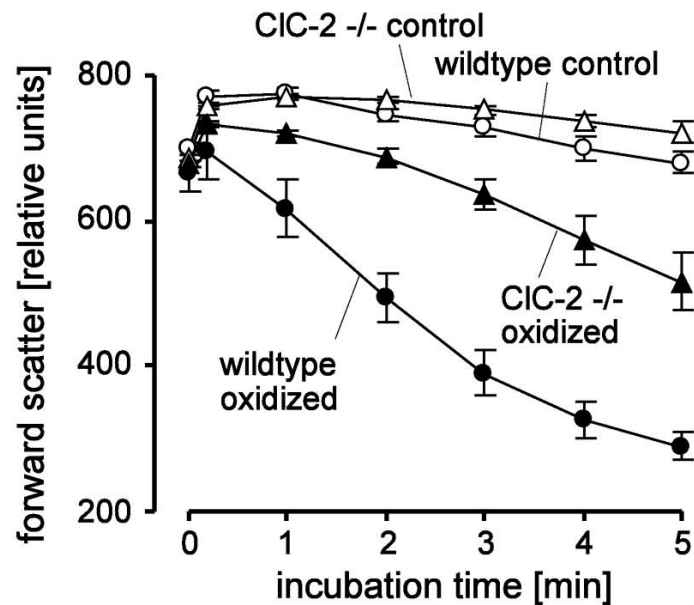
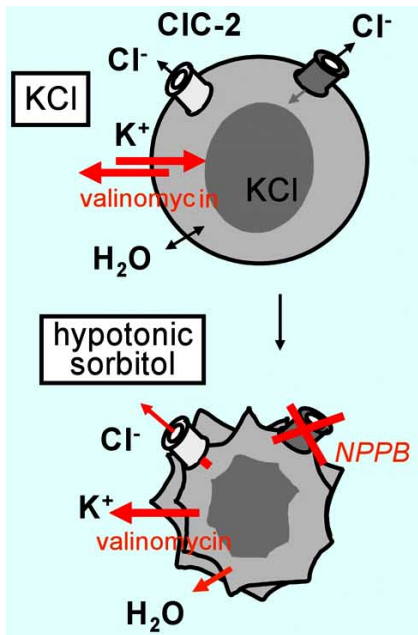
In oocytes of the nematode *C. elegans*, knockout of CLH-3, the putative orthologue of the human CIC-2, does not affect RVD compared to oocytes from control animals [32]. In that study the authors show that CLH-3 is activated during meiotic maturation and the channel could be identified as a critical component of intercellular communication between oocytes and the surrounding gonadal sheath cells. Similar function in intercellular communication has been demonstrated for CIC-2 in mammals. CIC-2 is crucially important for retinal and testinal function [18]. The *clcn2*<sup>-/-</sup> mice exhibit a degeneration of photoreceptors and

male germ cells leading to the total loss of both cell types in the adult animals. Both cell types are functionally dependent on supporting epithelial cells. Taken together, these findings strongly suggest that CIC-2 has a pivotal function in physiological processes depending on heterologous cell-cell interactions, intercellular communication, and regulation of cell cycle-dependent processes.

## FUNCTIONAL CLC-2 EXPRESSION IN MAMMALIAN ERYTHROCYTES

A previous study in our laboratories [19] analyzed functional CIC-2 expression in mature mammalian erythrocytes by the comparison of erythrocytes from wildtype and *clcn2*<sup>-/-</sup> mice [33]. For these experiments, KCl-equilibrated and K<sup>+</sup>-permeabilized erythrocytes from wildtype and *Clcn2*<sup>-/-</sup> mice were suspended in hypotonic sorbitol solution in the presence of the anion channel (but not CIC-2 inhibitor) 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB; 50 μM) and cell volume changes were analyzed by flow cytometry. In this setting, the volume decrease (by loss of KCl and H<sub>2</sub>O) of the initially swelled erythrocytes exclusively depends on the activity of NPPB (50 μM)-insensitive Cl<sup>-</sup> channels. The data indicated very low but significant resting activity of CIC-2 in control erythrocytes. Oxidative stress stimulated fast erythrocyte shrinkage which was extremely significantly more pronounced in wildtype as compared to *clcn2*<sup>-/-</sup> erythrocytes (Fig. 2). Activation of CIC-2 by oxidative stress was further confirmed for CIC-2 channels heterologously expressed in *Xenopus laevis* oocytes [19] indicating that redox potential-triggered signaling events that activate ion channels also occur in nucleated cells. Finally, CIC-2 protein expression was shown in human erythrocyte ghosts by immunoblotting [19]. In summary, CIC-2 is expressed in human and mouse erythrocytes. Cell swelling-induced activation, in contrast to nucleated cells, requires oxidative stress-stimulated transition of a dormant and inactive into an activatable channel.

This oxidative stress-stimulated activity of CIC-2 hints to an involvement of the channel in physiological processes associated with the response to oxidative stress. Among those is the execution of the suicidal death program [34-37]. Oxidatively injured erythrocytes delay hemolytic cell death by shrinking, blebbing of cytosol-filled membrane vesicles and presentation of "eat-me" signals at the surface. Specifically, oxidative insults induce Ca<sup>2+</sup> entry into human erythrocytes through TRPC6 and other cation channels [38] and subsequent activation of Gardos K<sup>+</sup> channels followed by erythrocyte membrane hyperpolarization and cellular loss of KCl and osmotically obliged H<sub>2</sub>O [39]. Shrinkage, in turn, further stimulates cation channel activity leading to sustained elevated cytosolic free Ca<sup>2+</sup> concentrations which trigger proteolytic cleavage of the cytoskeleton and phospholipid scrambling. The former leads to membrane blebbing and hence to portioning of the erythrocytes, the latter - to phosphatidylserine exposure at the surface. Phosphatidylserine is a recognition signal which fosters engulfment of the dying erythrocytes by macrophages prior to their hemolysis. This suicidal death program prevents the release of hemoglobin into the blood stream and precipitation of hemoglobin in the acid renal tubules which may give rise to acute renal failure.



**Fig. (2). Functional expression of CIC-2 in erythrocytes.** Oxidation-induced cell-shrinkage of cation-permeabilized (4  $\mu$ M gramicidin D, 2  $\mu$ M valinomycin) erythrocytes. Shown are cell-volume changes upon suspension of KCl-equilibrated mouse erythrocytes in K<sup>+</sup>- and Cl<sup>-</sup>-free solution containing the chloride channel blocker NPPB (50  $\mu$ M). This maneuver induced cell shrinkage by loss of cell KCl and water, which was dependent mainly on a NPPB-insensitive Cl<sup>-</sup> conductance. Mean forward scatter ( $\pm$ S.E. of n = 4 mice in each group) was determined by flow cytometry analysis as a measure of cell volume. Data are given for control (open symbols) and oxidized (*tert*butylhydroperoxide, 1 mM for 15 min; closed symbols) erythrocytes taken from wildtype (circles) and *clcn2*<sup>-/-</sup> mice (triangles).

Stress-induced loss of KCl, cell shrinkage and execution of the suicidal erythrocyte death program is attenuated by anion channel inhibitors. NPPB (100  $\mu$ M) inhibits only about 50% of the oxidative stress-induced phosphatidylserine exposure [40]. It is tempting to speculate that oxidation-stimulated NPPB-insensitive anion channels such as CIC-2 contribute to cellular loss of KCl and cell shrinkage.

A further oxidative stress-stimulated process is the release of ATP by mammalian erythrocytes [41]. Various stress stimuli such as hypoxia/hypercapnia or mechanical deformation have been demonstrated to stimulate ATP release by erythrocytes. This process requires cAMP and CFTR activity. Erythrocyte-derived ATP induces relaxation of blood vessels via the formation of NO by endothelial cells. NO in turn inhibits ATP release from erythrocytes. By those mechanisms, erythrocytes sense the oxygen tension and regulate the vascular resistance. Impaired release of ATP by erythrocytes results in primary pulmonary hypertension highlighting the importance of erythrocyte ATP release for arterial smooth muscle control [42]. To date, it is not clear whether or not oxidation-induced activation of erythrocyte CIC-2 channels has any functional significance for the oxidation-induced ATP release. The observed modification of CIC-2 by ATP/AMP via binding to the CBS domain [14, 15], however, might suggest that ATP release may alter CIC-2 channel function.

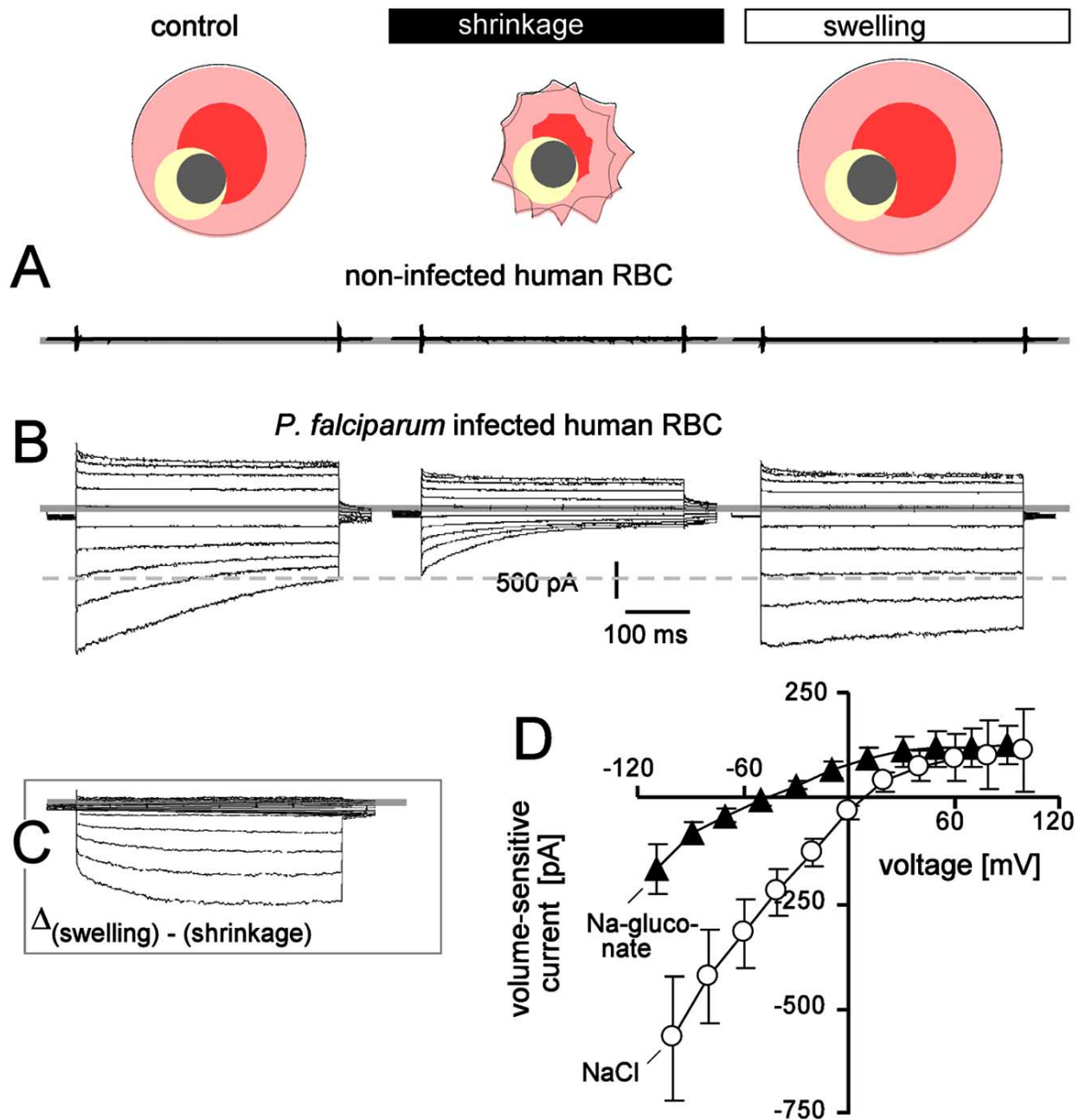
#### ACTIVATION AND FUNCTIONAL SIGNIFICANCE OF CLC-2 CHANNELS IN MALARIA-INFECTED ERYTHROCYTES

The intraerythrocytic amplification of the malaria parasite *Plasmodium falciparum* induces new pathways of

solute permeability in the host cell membrane [43,44]. These so called *New Permeability Pathways* are organic osmolyte and ion channels which play a pivotal role for parasite development by supplying the parasite with nutrients, disposing of metabolic waste and organic osmolytes, adapting the host's electrolyte composition to the parasite's needs, and lowering the colloid osmotic pressure of the host erythrocyte [45]. The latter function avoids pre-mature hemolysis of the host cell [46].

The number of pathways (and their nature – erythrocyte- or parasite-derived) that form the altered permeability of infected erythrocytes is highly controversially debated [47]. The intraerythrocytic parasite confers high oxidative stress to its host cell [48] and oxidative processes contribute to the induction of the altered erythrocyte membrane permeability [41, 49]. Therefore, we tested for malaria-induced activation of CIC-2 in the host membrane. Whole-cell currents of *Plasmodium falciparum*-infected human erythrocytes and of *P. berghei*-infected mouse erythrocytes were recorded in our laboratory under control conditions and during osmotic cell swelling and shrinkage [19]. The data clearly demonstrate that in infected but not uninfected cells, cell shrinkage inhibits and cell swelling activates CIC-2 currents (Figs. 3 and 4) [19,50].

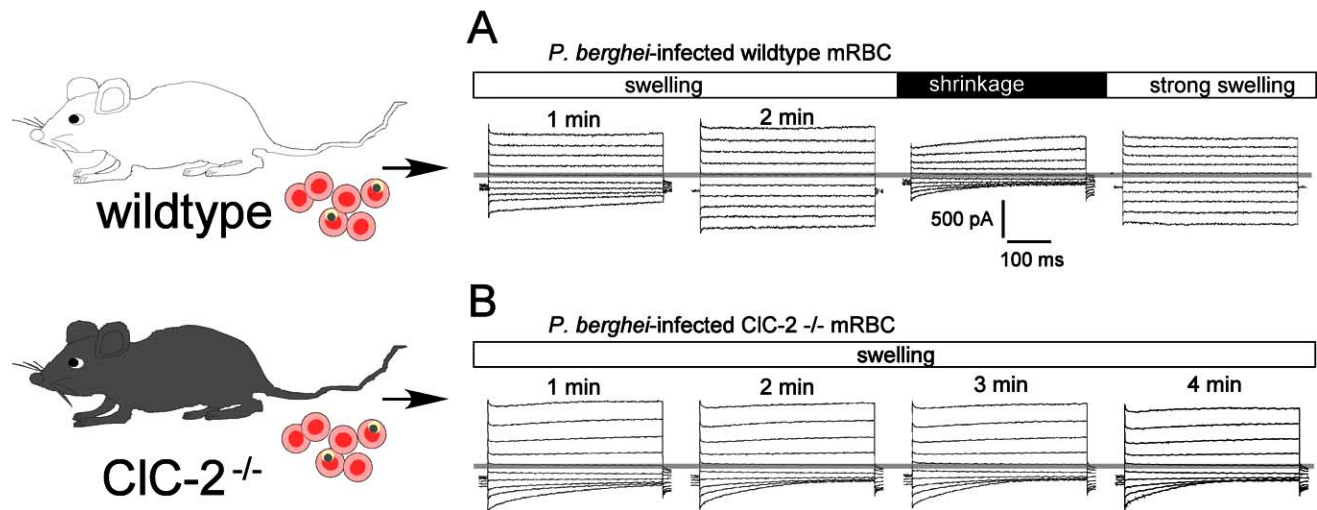
The observed swelling-induced activation of CIC-2 Cl<sup>-</sup> channels in *Plasmodium*-infected human and mouse erythrocytes points to a possible role of this channel in RVD. Thus, CIC-2 deficiency may impair cell volume regulation of infected erythrocytes leading to an increase in host volume. To test this possibility, our laboratory determined the cell volume of erythrocytes drawn from *P. berghei*-infected *clcn2*<sup>-/-</sup> and wildtype mice by forward scatter in flow cytometry. As a result, uninfected and infected *clcn2*<sup>-/-</sup>



**Fig. (3).** *P. falciparum* induces cell volume-sensitive anion channels in human erythrocytes. **A** and **B**, whole cell current traces recorded (**A**) in a non-infected and (**B**) in a late trophozoite-infected human erythrocyte in isotonic (control, *left*), hypertonic (shrinkage, *middle*), and hypotonic (swelling, *right*) NaCl bath solution combined with NaCl pipette solution. Currents were evoked by 10 voltage pulses (400 ms each) from -10 mV holding potential to voltages between -100 mV and +80 mV. Applied voltages refer to the cytoplasmic face of the membrane with respect to the extracellular space. Inward currents, defined as entry of positive charge from the extracellular space into the cytosol, are negative currents and depicted as downward deflections of the original current traces. Zero current is indicated by *gray line*. *Dotted grey line* indicates the peak inward current in the shrunken cell. **C**, volume sensitive current fraction as calculated by subtracting the current traces in **B** obtained during cell shrinkage from those during cell swelling. **D**, current-voltage (I/V) relationships of the volume-sensitive current fraction in infected human erythrocytes recorded as in **B** with NaCl (*open circles*) and sodium D-gluconate pipette solution (*closed triangles*). Data are means  $\pm$  SE, n = 5-11.

erythrocytes exhibited a significantly higher forward scatter than the corresponding wildtype erythrocyte groups (Fig. 5) [19]. The CIC-2 current fraction of infected mouse and human erythrocytes is  $ZnCl_2$  sensitive ( $IC_{50}$  in the range of 100  $\mu M$ ) but NPPB-insensitive [19]. Further experiments studying the effect of CIC-2 inhibition by  $ZnCl_2$  indicate a

significant increase in forward scatter of infected wildtype erythrocytes but not of uninfected wildtype or uninfected and infected *clcn2*<sup>-/-</sup> erythrocytes (Fig. 5) [19]. Thus, inhibition of CIC-2 increases cell volume only in parasitized wildtype erythrocytes, suggesting the functional significance of CIC-2 in the cell volume maintenance of the infected host



**Fig. (4).** CIC-2-dependent inwardly rectifying anion currents in mouse erythrocytes. **A-B**, whole cell current traces recorded at different time points (as indicated) in a *P. berghei*- (late trophozoite)-infected wild-type (**A**), and an infected *clcn2*<sup>-/-</sup> mouse erythrocyte (**C**). Continuous cell swelling was induced by combining a hypertonic pipette solution (170 mM NMDG-Cl) with an isotonic bath solution (140 mM NMDG-Cl). In addition, in (**A**) cell shrinkage and further strong cell swelling was evoked by increasing and decreasing the bath osmolarity by 450 and 150 mosM, respectively.

erythrocytes. Despite this apparent function, *P. berghei* develops well in *clcn2*<sup>-/-</sup> mice [19] suggesting that other infection-induced anion channels (see below) can substitute for CIC-2.

To search for a functional significance of CIC-2 in *malaria tropica* we sequenced all 24 CLCN2 exons of malaria patients from a Central African population in order to find naturally occurring gene variants in a human population. Six single amino acid exchanges in the intracellular N-terminus (P48R, R68H), in the pore domain (G199A), or in the intracellular C-terminus (R646Q, R725W, R747H) were identified at low frequency (Fig. 1). Heterologous expression of these polymorphisms in *Xenopus laevis* oocytes revealed altered CIC-2 function [51]. The heterozygous carriers of these polymorphisms did not show a frequency or a course of malaria infection which diverged from the cross-section of population (unpublished own data). Hence, a functional role of CIC-2 in human malaria cannot be deduced from these experiments.

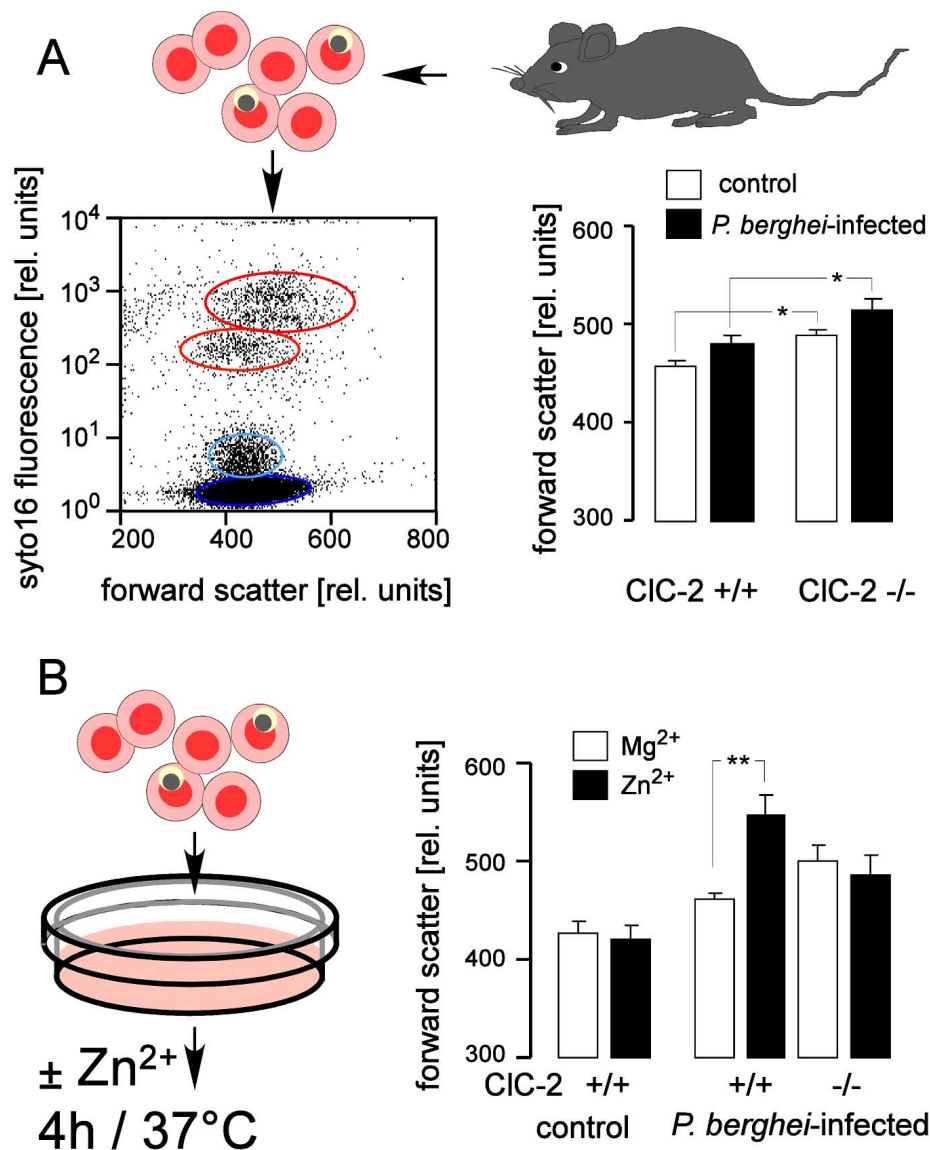
#### IS CLC-2 THE PUTATIVE PLASMODIUM FALCIPARUM-ENCODED ERYTHROCYTE SURFACE ANION CHANNEL (PSAC)?

In 2000, Sanjay Desai and coworkers in Bethesda (Maryland) employed for the first time the whole-cell and on-cell patch-clamp techniques to characterize the altered membrane conductance of *P. falciparum*-infected erythrocytes [52]. By on-cell single channel recording the same and later studies (see below) identified an anion channel with low unitary conductance (<3 pS) in the membrane of trophozoite-stage infected human erythrocytes. To analyze the single channel properties, Desai and co-workers applied very high Cl<sup>-</sup> concentrations. At such extremely high concentrations of the charge carrier (1.1 M Cl<sup>-</sup>) the unitary conductance has been assumed to increase to 20 pS. It was estimated to be present at 1000–2000 functional copies per

infected erythrocyte. Since it was not observed in uninfected erythrocytes this channel type has been called the *plasmodium* erythrocyte surface anion channel (PSAC). Although having a linear current voltage relationship, PSAC shows highly voltage-dependent single channel gating with higher open probability at hyperpolarizing voltages. This voltage-dependence correlates well with the observed infection-induced inwardly rectifying whole-cell currents. PSAC is inhibited by furosemide (125 μM) added to the pipette solution and shows a complex fast flickering gating with bursts of openings with mean open times of 0.5 ms. Consistent with this complex gating, the spectral density of an on-cell recording revealed a 1/f profile (a channel with simple, non-bursting gating would have a Lorentzian spectral density). Spectral analysis of the infection-induced whole-cell currents also revealed a 1/f profile.

These parallel power spectrum profiles indicate that the PSAC single-channel type seen with on-cell patch-clamp recording can fully account for the whole-cell currents. Because of this observation combined with the voltage-dependent gating and the furosemide sensitivity of both the infection-induced whole-cell currents and the PSAC channels, Desai *et al.* have concluded that PSAC is the predominant conductive Cl<sup>-</sup> pathway in the infected erythrocyte membrane. A further rationale for PSAC being the malaria-induced osmolyte channel of infected erythrocytes is the observation that the malaria parasites are rapidly killed by dantrolene derivatives. Dantrolene was considered specific for PSAC [53,54]. A more recent study, however, revealed that dantrolene inhibits at least one further type of anion channels activated by the intraerythrocytic parasite [47].

Meanwhile, the paradigm of a unique and parasite-derived organic osmolyte and anion channel with broad substrate specificity has been challenged by the observations of several independent groups. The group of Stéphane Egée and Serge Thomas showed that PSAC channels generate at physiological ionic strength only 20% of the inwardly



**Fig. (5). Functional significance of the  $\text{Zn}^{2+}$ -sensitive CIC-2-dependent anion channels in *P. berghei*-infected mouse erythrocytes. A, left. Dot blot recorded by flow cytometry showing fluorescence intensity of the DNA-specific dye syto16 against forward scatter of erythrocytes from infected mice. Non-infected erythrocytes and reticulocytes are outlined dark and light blue, erythrocytes infected with ring- and trophozoite/schizont stages of *P. berghei* are outlined in light and dark red, respectively. A, right. Forward scatter reflecting cell volume (mean  $\pm$  S.E.) of non-infected (i.e. low syto16 fluorescence) and *P. berghei*-infected (i.e. high syto16 fluorescence) erythrocytes from wildtype (n = 17) and *clcn2*<sup>-/-</sup> mice (n = 10). B, Forward scatter was recorded in non-infected and *P. berghei*-infected mouse erythrocytes post-incubated for 4 h at 37 °C in the presence of  $\text{ZnCl}_2$  or  $\text{MgCl}_2$  (1 mM each).**

rectifying anion current in trophozoite-infected erythrocytes [55]. At physiological ionic strength, most of the whole-cell current in infected erythrocytes is carried by erythrocyte-derived 18 pS anion channels [55-60]. Moreover, stimulating infected cells with micromolar concentrations of serum albumin induces a further erythrocyte-derived anion channel with outwardly rectifying current-voltage relationship and 80 pS single channel conductance [47,49,61-63]. This strongly suggests that under physiological conditions, i.e., in the plasma which contains about 700  $\mu\text{M}$  serum albumin, PSAC generates only a very small fraction of the infection-induced conductance. Furthermore, the 18 pS and 80 pS anion channels conduct molecules as large as ATP [64]. For the latter channel (but not for PSAC), an interference of lactate and  $\text{Cl}^-$  permeation with neutral organic osmolytes has been

demonstrated [50] suggesting that the 80 pS channel is an organic osmolyte channel.

In accordance with these electrophysiological findings, Henry Staines demonstrated that the solute transport by *P. falciparum*-infected cells is not consistent with a simple single channel model [65]. Similarly, Gottfried Lisk in Sanjay Desay's laboratory reported that transport of different solutes through the infected erythrocyte membrane exhibited different pharmacologies [66]. To remain consistent with a single channel model, two distinct mechanisms of transport through the PSAC has been postulated [67].

Circumstantial evidence for a parasite-derived origin of the PSAC came from the observations that i) PSAC is functionally conserved in divergent malaria parasites [68], ii) two

distinct parasite isolates, grown in erythrocytes from a single donor, exhibit channel activity with measurably different voltage-dependent gating [69], iii) a mutation in *P. falciparum* conferring blasticidin S-resistance was linked to altered gating, pharmacology, selectivity profile or occurrence of sub-conductance states of the PSAC channel in single channel recording [70] iv) changes in PSAC reduce leupeptin uptake and can confer drug resistance in infected erythrocytes [71].

All these conclusions, however, are based on the assumption that PSAC is the only channel in infected erythrocytes. Human erythrocytes, in contrast, reportedly express (besides CIC-2) CFTR [72-76], 18 pS-, 80 pS-, 300 pS-, [55,58-60] and acid-sensitive anion channels [77], Gardos K<sup>+</sup> channels [78], TRPC6 [38] and possibly TRPC3 [79], Pannexin-1 [80] and P2X7 cation channels and tetrodotoxin-sensitive Na<sup>+</sup> channels [81] and most probably numerous further yet unidentified ion channel types. Besides PSAC at least four other channel types are active in infected cells [44]. The experimental settings of the above mentioned studies on the parasite-derived origin of PSAC were not appropriate to experimentally dissect these individual current fractions and unitary conductance transitions, respectively. For instance, differences in whole-cell outward current between erythrocytes infected with two different parasite isolates [69] might simply be due to a different activity of the outwardly-rectifying 80 pS anion channels rather than to a parasite isolate-specific voltage dependence of PSAC. Along those lines, variations in the fractional activities of two or more different channel types might simply be misinterpreted as the postulated parasite mutant-linked alterations in PSAC single channel properties [70]. As a matter of fact, the different ion channels are slowly activated during parasite development [41,62], and the time course of activation may differ between the different ion channel types. If so, the composition of active ion channel types should differ between erythrocytes parasitized with fast growing parasites and those infected with slowly developing mutants which confer lower cellular stress to their hosts. Moreover, cross-breeding of putative "PSAC-mutant" with wildtype parasites failed to show direct inheritance of PSAC properties [82].

Taken together, the rationale for the model is very weak in which a parasite-derived PSAC channel which in fact comprises only a minor fraction of the overall infection-induced channel activity is underlying the altered erythrocyte membrane permeability while the major fraction of erythrocyte-derived 18 pS and 80 pS anion channels (for which lactate, neutral organic osmolyte and/or ATP permeability have been confirmed experimentally) do not contribute to the altered permeability. Finally, until now, PSAC has not been found in the parasite genome which might hint to the possibility that PSAC doesn't exist.

But what is PSAC if not a parasite-derived channel? A potential answer is suggested by the observation that "PSAC" increasingly activates with increasing ionic strength (i.e., so more the experimental situation differs from physiological conditions) [55]. Hyperosmolar whole-cell recording perturbs the cell volume of the recorded cell even when applying the same osmolarity in bath and pipette (cells pre-shrink in hyperosmolar bath solution and re-swell upon dialysis of the cytosol by the hyperosmolar pipette solution

after break-in into the whole cell mode. The other way round, when held in isotonic bath solution, the cells swell upon break-in into the whole-cell mode and re-shrink upon switching to hyperosmolar bath solution). Most notably, "PSAC" activity at high osmolarity is inhibited by zinc but is relatively insensitive to furosemide and NPPB [55]. Volume stress-associated activity, zinc sensitivity, relative furosemide and NPPB-insensitivity, inward rectification and very low single channel conductance, however, is highly reminiscent to CIC-2, strongly suggesting that the PSAC phenomenon is generated by CIC-2 channels and further suggesting that CIC-2 is not involved in the NPPB- and furosemide-sensitive organic osmolyte permeability of *P. falciparum*-infected erythrocytes.

## CONCLUDING REMARKS

CIC-2 channels are functionally expressed in mammalian mature erythrocytes. Under control conditions, CIC-2 channels are dormant. Once activated by oxidative stress or by the intraerythrocytic malaria parasite, they depend on cell volume and contribute to RVD of the parasitized erythrocyte. In uninfected erythrocytes, CIC-2 may delay hemolysis of oxidatively injured erythrocytes in concert with Gardos K<sup>+</sup> and TRPC6 nonselective cation channels. Concomitant activation of CIC-2 may contribute to cellular loss of KCl and osmotically obliged H<sub>2</sub>O and cell shrinkage. Sustained erythrocyte shrinkage triggers the suicidal death program of erythrocytes which fosters their clearance by phagocytes prior to their detrimental hemolysis.

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