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ASSOCIATION OF THE BAND 3 PROTEIN WITH A VOLUME-ACTIVATED, ANION AND AMINO ACID CHANNEL: A MOLECULAR APPROACH

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Summary

In response to swelling, cells recover their initial volume by releasing intracellular solutes *via* volume-sensitive pathways. There is increasing evidence that structurally dissimilar organic osmolytes (amino acids, polyols, methyl amines), which are lost from cells in response to swelling, share a single pathway having the characteristics of an anion channel. However, the molecular identity of this pathway remains to be established. It has been suggested that the erythrocyte anion exchanger (AE1) or some AE1-related proteins could be involved. A direct evaluation of this possibility has been made by comparing the functional properties of two AE1s when expressed in *Xenopus laevis* oocytes: tAE1 is from a fish erythrocyte which releases taurine when swollen, and mAE1 is from a mammalian erythrocyte which does not regulate its volume when swollen. While mAE1 performs exclusively Cl^-/Cl^- exchange, tAE1 behaves as a bifunctional protein with both anion exchange and Cl^-/taurine channel functions. Construction of diverse tAE1/mAE1 chimaeras allows the identification of protein domains associated with this channel activity. Thus, some AE1 isoforms could act as a swelling-activated osmolyte channel, a result having a potentially important implication in malaria. This review also discusses the possibility that several different proteins might function as swelling-activated osmolyte channels.

Key words: cell volume regulation, organic osmolytes, taurine transport, osmoregulation, chloride channels, band 3 protein, anion exchanger, erythrocytes.

Introduction

The regulation of volume in response to changes in osmolality is a fundamental property of most cells. Following cell swelling in hypo-osmotic medium, cells tend to recover their initial volume by a process termed regulatory volume decrease (RVD). RVD is achieved by the loss of osmotically active intracellular compounds accompanied by an osmotic loss of water. In most cells, inorganic ions (mainly K^+ and Cl^-) play a central role in the RVD response, usually leaving from the cell *via* a K^+/Cl^- cotransport system and/or separate volume-activated channels (reviewed by Hoffman and Simonsen, 1989; Sarkadi and Parker, 1991).

However, the cytosol of all organisms contains at high concentrations (tens to hundreds of millimolar) small, organic, osmotically active solutes which have cytoprotective functions (Wright *et al.* 1986). These compounds belong to different chemical classes: polyols (such as sorbitol or myo-inositol), amino acids (such as taurine) and methyl amines (such as betaine). Their accumulation in cells is due to synthesis from metabolic precursors or is mediated by energy-dependent transport mechanisms (such as Na^+ or H^+ cotransporters). In all phyla investigated, from bacteria to humans, it has been observed that such solutes are lost in response to cell swelling, demonstrating that they also play a key role in cell volume homeostasis. In fact, their release may account for as much as half of the total RVD (Garcia-Romeu *et al.* 1991). That loss occurs *via* specific pathway(s) activated by the volume increase. In contrast to the situation with swelling-activated K^+ and Cl^- pathways, little is known about the nature and regulation of the volume-dependent organic solutes pathway(s). The results accumulated in the last few years are consistent with the hypothesis that the volume-activated transport of organic osmolytes is *via* a single pathway that resembles a channel and which is blocked by a range of anion transport inhibitors (Kirk *et al.* 1992; Roy and Malo, 1992; Strange *et al.* 1993). This particular sensitivity of the pathway to anion transport inhibitors led several authors simultaneously to propose a role in the swelling-activated response for band 3, the red cell electrically neutral Cl^-/HCO_3^- exchanger AE1, either as the swelling-activated transport system (Goldstein and Brill, 1991) or as a regulatory protein (Garcia-Romeu *et al.* 1991; Motais *et al.* 1991, 1992). The proposal of Goldstein and Brill (1991) was essentially based upon studies showing a close relationship in the dose–response curves for the effect of 4,4′-diisothiocyanato-stilbene-2,2′-disulphonic acid (DIDS) upon anion exchange and volume-activated transport of organic solutes, suggesting that band 3 acts as the swelling-dependent transporter. The proposal of Motais and colleagues was based upon studies showing that hypotonic swelling

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activates not only amino acid (taurine) and K^+ efflux but also pathway(s) allowing solutes as diverse as Na^+, choline or tetramethylammonium to diffuse passively across the cell membrane. DIDS and other blockers of the anion exchanger fully inhibit the movement of all these solutes. Moreover, the dose–response curves for the effect of DIDS on swelling-activated transport of K^+, taurine, Na^+ and choline are superimposable. It was suggested that it is unlikely that these compounds all use the same pathway, and it was proposed instead that DIDS and other anion inhibitors interact with a single target, the band 3 anion exchanger that controls the activity of multiple transport systems, rather than with the transport systems themselves.

Several recent reviews have analyzed in detail the main results leading to the concept that the swelling-activated loss of organic solutes may occur *via* a single pathway having the characteristics of a channel (Strange, 1994; Strange and Jackson, 1995; Strange *et al.* 1996). In the present review, after a brief summary of these data, we examine the evidence for the involvement of the erythrocyte band 3 anion exchanger (AE1) in the volume-dependent transport of osmolytes.

Characteristics of the volume-activated, organic osmolyte efflux pathway

Evidence for a Cl^- channel

Swelling-activated organic osmolyte efflux has been observed in cells from most phyla, but characterization of the efflux pathways has been carried out mainly in mammalian cells (renal epithelial cells, glial cells, human lung cells, lymphocytes and Ehrlich ascites cells) and in cells of lower vertebrates (fish erythrocytes and hepatocytes). These studies demonstrate that swelling-activated effluxes of amino acids, polyols and methylamines share a number of characteristics. (1) They are mediated by passive, Na^+-independent, very low-affinity routes (Fincham *et al.* 1987; Fugelli and Thoroed, 1986; García *et al.* 1991; Siebens and Spring, 1989; Sanchez-Olea *et al.* 1991). (2) The transport pathways do not saturate with substrate concentrations up to $100\,mmol\,l^{-1}$ (Haynes and Goldstein, 1993; Kirk *et al.* 1992; Roy and Malo, 1992; Strange *et al.* 1993). (3) The similarities of the amino acid, polyol and methylamine losses observed in diverse organisms or after various treatments that inhibit or stimulate swelling-induced efflux led to the proposal that a single transport pathway mediates the efflux of these structurally unrelated organic osmolytes (Kirk *et al.* 1992; Strange *et al.* 1993). (4) From its pharmacological profile, it was then suggested that this pathway is a swelling-activated anion channel (Kirk *et al.* 1992; Roy and Malo, 1992), a hypothesis subsequently supported by a series of quantitative comparisons of dose–response curves, concentration-dependence and selectivity for different inhibitors and substrates in several cell types (Goldstein and Davis, 1994; Kirk and Kirk, 1993, 1994; Joyner and Kirk, 1994; Lewis *et al.* 1996).

More direct evidence for the involvement of anion channels was first presented by Banderali and Roy (1992): using single-channel patch-clamp analysis, they demonstrated that some amino acids, which appear to play a role in RVD in cultured kidney cells (MDCK), could permeate a swelling-activated anion channel in this cell line. Subsequent comparisons of electrophysiological measurements and isotope flux assays performed on C6 glioma cells and on other tissues (reviewed in Strange and Jackson, 1995) provide additional evidence in support of the hypothesis that a swelling-activated anion channel mediates the efflux of multiple organic osmolytes from cells.

This swelling-activated anion channel possesses the following characteristics: it is an outwardly rectifying conductance, i.e. the current–voltage relationship is not linear, the slope of the curve becoming steeper as the cell interior is rendered more positive. It generally exhibits a high permeability (P) for anions over cations (P_{cat}/P_{Cl}<0.03–0.05), but has a broad anion specificity with the permeability sequence: $SCN^- \approx NO_3^- > I^- > Br^- > Cl^- > F^- >$ isethionate > gluconate. The single-channel conductance is 40–50 pS at +120 mV (Strange *et al.* 1996). Swelling-activated anion currents are inactivated by membrane potentials above +60 mV (cell interior positive) and are blocked by extracellular nucleotides such as ATP and cyclic AMP at high concentrations. The conductance is inhibited by a variety of agents including DIDS and other stilbene derivatives, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), tamoxifen, dideoxyforskolin, lanthanum, polyunsaturated fatty acids and ketoconazole. However, the drug sensitivity can vary between cell types.

Regulation of the channel

Cell swelling is a necessary condition to activate the channel, but activation is also critically dependent on some other parameters, mainly the cellular electrolyte levels. Thus, in trout erythrocytes, the channel, which is activated by hypotonic-induced swelling, is not activated when a volume increase of similar magnitude is brought about by raising cytoplasmic NaCl (*via* a parallel functioning of Na^+/H^+ and Cl^-/HCO_3^- antiporters) or NH_4Cl levels. In this situation, erythrocytes adopt another strategy to regulate their volume: they no longer lose taurine or other organic osmolytes, but instead lose exclusively K^+ and Cl^- *via* a K^+/Cl^- cotransporter (Motais *et al.* 1991). Thus, the intracellular ionic strength determines which regulatory pathway (channel or cotransport) is activated in response to swelling. Recently, in patch-clamp studies of skate hepatocytes, Jackson *et al.* (1996) demonstrated that the channel activation is inversely related to intracellular Cl^- concentration. Thus, intracellular ionic strength and/or $[Cl^-]$ play an important role in modulating the channel activity. As suggested by Strange (1994), such a modulation could have important physiological implications: if swelling is induced by electrolyte accumulation, it would be advantageous for the cells to regulate their volume by losing electrolytes rather than organic osmolytes, which are metabolically expensive.

Channel activation also requires intracellular ATP, but it

Table 1. *Comparison of functional properties for wild-type anion exchangers (mouse AE1=mAE1 and trout AE1=tAE1) and two chimaeras (TmT and MZM)*

	Wild types		Deletion mutants	Trout *versus* mouse chimaeras	
	MmM*	TZT*	tAE 1ΔN	TmT	MZM
Expressed proteins					
	* MmM = mAE1	* TZT = tAE1			
Functional properties					
Cl^-/Cl^- exchange	+	+	+	+	+
Cl^- conductance	−	+	+	−	−
Taurine transport	−	+	+	−	−
			Channel activity is independent of cytoplasmic tail	Z-loop is necessary for channel activity	Z-loop is not sufficient for channel activity

Data are taken from Fiévet *et al.* (1995).

seems that ATP hydrolysis or phosphorylation reactions are not required (Jackson *et al.* 1994).

Which specific protein(s) may be involved in this response?

Cells possess several types of volume-sensitive anion channels, mainly a voltage-gated Cl^- channel, Cl^--C2, recently cloned (Thieman *et al.* 1992), and a large-conductance or 'maxi' Cl^- channel. These channels do not seem to mediate organic solute movements and have very different electrophysiological characteristics. The channel responsible for swelling-activated organic osmolyte efflux has been termed VSOAC (for Volume-Sensitive Organic osmolyte, Anion Channel) but its molecular identity remains to be established. A protein recently cloned from dog kidney cells could be either the VSOAC channel or a regulator of this channel (discussed below). However, as discussed above, it has also been proposed that the band 3 protein, which is an electroneutral exchanger and *not* a channel, is involved in swelling-activated organic osmolyte efflux.

In oocytes, red cell band 3 generates a Cl^- channel mediating taurine transport

The putative role of band 3 in the volume-dependent response, either as a transport system or as a regulatory protein, has been investigated using a molecular approach. Fish erythrocytes respond to osmotic swelling by undergoing RVD: this involves the efflux of both KCl and amino acids, primarily taurine (Fincham *et al.* 1987; Fugelli and Thoroed, 1986; Garcia-Romeu *et al.* 1991). By contrast, anucleated mammalian erythrocytes do not show swelling-activated taurine transport, volume regulation being achieved by activation of K^+/Cl^- cotransport, which occurs to a variable extent in different species (reviewed by Lauf *et al.* 1992). However, both fish and mammalian erythrocytes possess the band 3 anion exchanger (AE1). We have cloned the trout erythrocyte AE1 and compared its functional properties with those of mouse erythrocyte AE1 when both proteins are expressed in *Xenopus laevis* oocytes (Fiévet *et al.* 1995).

Trout AE1 (tAE1) and mouse AE1 (mAE1) are highly homologous proteins. Most of the differences are located in two distinct domains: the N-terminal cytoplasmic fragment and an extracellular loop linking transmembrane fragments 5 and 6. This loop is large in tAE1 (Z-loop) and short in mAE1 (m-loop). It is inserted near the site of covalent binding of DIDS. When expressed in *Xenopus* oocytes, both isoforms, as expected, elicit anion exchange activity (Table 1; Cl^-/Cl^- exchange). Unexpectedly for an electrically neutral exchange, however, tAE1 expression also causes a dramatic increase in oocyte Cl^- conductance. This Cl^- conductance has a quasi-linear current–voltage relationship, is not inactivated by depolarisation and is correlated in a non-linear, sigmoidal fashion to the amount of the expressed proteins. tAE1 expression also induces a large increase in Na^+-independent taurine permeability which is linearly correlated to Cl^- conductance. In contrast, expression of mAE1 induces neither Cl^- conductance nor taurine transport. Taken together, these data suggest that tAE1 is capable of functioning as an anion/organic osmolyte channel. Conversely, a close isoform derived from a cell that does not regulate its volume in response to swelling, mAE1, cannot function as an osmolyte channel.

A series of mutants and chimaeras constructed from tAE1 and mAE1 allows the identification of some protein domains associated with these functional differences (Table 1). Removal of the N-terminal cytoplasmic fragment, a domain

that links the band 3 protein to the cytoskeleton, does not affect the capacity of tAE1 to function as an osmolyte channel. In contrast, the large extracellular Z-loop is necessary (but not sufficient) for expression of both channel activity and taurine transport.

Is red cell band 3 a channel or a channel regulator?

The results reported above indicate that the trout AE1, when expressed in oocytes, functions spontaneously as an anion/organic osmolyte channel (which in the native erythrocyte is activated only by cell swelling). There are two explanations of why some isoforms of AE1 are capable of channel activity.

First, it is generally recognised (Passow, 1986) that, in red cells, monomers of all AE1 isoforms mediate not only a highly efficient, electroneutral anion exchange but also a very small diffusive anion flow, this Cl^- conductance determining the red blood cell resting potential. From structural analysis, it appears that several segments of the protein form a channel that extends across the membrane. Substrate anions (i.e. Cl^-, HCO_3^-) are accumulated at the channel orifice by a cluster of fixed positive charges. The step that subsequently opposes diffusion and imposes an electrically neutral anion exchange is assumed to be confined to a narrow portion of the channel. According to the different models ('lock in' or 'knock on'), one pair or several pairs of fixed charges, located on distinct adjacent segments of the peptide chain, occlude the channel and form a gate. The penetrating anion 'opens' the gate either by forming a complex with the paired fixed charges, the complex then catalyzing a change from an inward- to an outward-facing conformation and *vice versa* ('lock in' model), or by breaking a series of salt bridges formed by each pair of fixed charges ('knock on' model). The small conductive component is explained by the rare occurrence of either the anion jumping out of the substrate–protein complex before the paired fixed charges undergo their conformational transition ('lock in' model) or the simultaneous opening of both pairs of fixed charges ('knock on' model).

A channel activity can then be induced in AE1 by any uncoupling of the paired fixed charges, leaving the channel free for diffusion. Such uncoupling can occur as a result of a conformational change in the channel-forming domain. It must be pointed out (1) that DIDS is known to bind at the orifice of the channel and to plug it; (2) that trout AE1, which is DIDS-sensitive in erythrocytes, loses that sensitivity when transfected in *Xenopus* oocytes and simultaneously expresses a channel activity (Fiévet *et al.* 1995), so that trout AE1 must adopt a different conformation in oocytes and in erythrocyte membranes; and (3) that the large Z-loop extends into the channel orifice of trout AE1. Shortening of this loop (chimaera TmT, see Table 1) is sufficient to induce both a recovery of DIDS-sensitivity and the disappearance of channel activity (Fiévet *et al.* 1995). Taken together, these results suggest that the large Z-loop confers some flexibility to trout AE1, allowing the channel to be enlarged and to be available for solute diffusion in response to erythrocyte swelling (or to adopt this enlarged configuration spontaneously in the oocyte environment).

An alternative explanation of how AE1 may be capable of channel activity is suggested by the data of Musch *et al.* (1994) for skate erythrocytes, where osmotic swelling promotes an increase in the number of band 3 tetramers. Thus, the osmolyte channel could be formed from aggregates of band 3. When trout AE1 is expressed in oocytes, the anion current is a sigmoidal (rather than linear) function of the level of band 3 expression (Fiévet *et al.* 1995), suggesting that the conductance pathway could be formed by multimeric complexes of the protein.

The view that an anion exchanger may also function as a channel is supported by a recent study performed with the triose phosphate/phosphate exchanger from the chloroplast (Schwarz *et al.* 1994). When reconstituted into an artificial membrane, this transporter, besides its normal counter-exchange mode, also functions as an anion-selective channel. Thus, it seems likely that trout AE1 possesses the capacity to function as a channel. However, an alternative possibility must also be considered. There is increasing evidence that some membrane proteins such as CFTR (cystic fibrosis transmembrane regulator) and multidrug resistance P-glycoprotein, acting independently of their own intrinsic transport activity, are able to regulate heterologous channels or transporters (Higgins, 1995; Hardy *et al.* 1995; Stutts *et al.* 1995).

Paulmichl *et al.* (1992) have cloned a small protein (255 amino acids), termed I_{Cln}, the expression of which in oocytes gives rise to an anion conductance that is constitutively active and possesses many characteristics of the current induced by VSOAC activation. Initially, I_{Cln} was considered to be a channel-forming protein; as the structural analysis suggested no transmembrane helices, it was proposed that the protein dimerises to form a porin-like channel consisting of eight antiparallel β-strands. This interpretation was subsequently called into question by Clapham and colleagues, who presented evidence first that *Xenopus* oocytes possess an endogenous swelling-activated anion current, termed $I_{Clswell}$, with characteristics identical to those induced by expression of I_{Cln} (Ackerman *et al.* 1994), and second that I_{Cln} is an acidic cytoplasmic protein, expressed widely, including in *Xenopus* oocytes (Krapivinsky *et al.* 1994). It was therefore proposed that I_{Cln} is not an anion channel but a channel regulator; overexpression of I_{Cln} in oocytes maintained in an isotonic medium resulting in the activation of the endogenous, volume-sensitive $I_{Clswell}$.

The I_{Cln} channel *versus* I_{Cln} regulator controversy is not yet resolved (see the next paragraph), but it prompted us to consider the possibility that expression of trout AE1 in oocytes results in the activation of the endogenous $I_{Clswell}$. If such an activation occurs, the AE1-induced current (I_{AE}) must display identical characteristics to those of $I_{Clswell}$. However, as illustrated in Table 2, a number of the characteristics tested so far are clearly distinct. Further, but still preliminary, data

Table 2. *A comparison of I_{AE1} and $I_{Clswell}$: properties that distinguish the AE1-induced Cl^- current (I_{AE1}) from the endogenous, swelling-activated Cl^- current ($I_{Clswell}$)*

	Anion conductance			Inhibited by			
Cl^- current	Collagenase treatment	6 days after defolliculation	Kinetics (voltage sensitivity)	Nucleotides	DIDS	Niflumic acid	Channel capacity for taurine
I_{AE1}	+	+		−	−	+	1
$I_{Clswell}$	−	−		+	+	−	4, 5

Data are from Fiévet *et al.* (1995).

The anion conductance was measured on oocytes devoid of follicle cells. Removal of follicle cells is achieved either by collagenase treatment or manually.

$I_{Clswell}$ is observed only after manual defolliculation; this channel activity decreases progressively as a function of time, disappearing 6 days after defolliculation.

Niflumic acid [2-(α,α,α trifluoro-*m*-toluidino)nicotinic acid] is a band 3 inhibitor.

Channel capacity for taurine: relative amount of taurine transported per unit of channel conductance.

indicate that I_{AE} and $I_{Clswell}$ are likely to represent distinct Cl^- conduction pathways.

In conclusion, at present, all the available data support the hypothesis that trout AE1 is a bifunctional protein with both anion exchange and osmolyte channel functions.

Is there a single type of VSOAC or are there multiple types?

In virtually all cells studied to date, swelling activates an outwardly rectifying anion conductance associated with organic osmolyte efflux. The characteristics of all these conductances are very similar, and it is implicit that they are due to the activity of a single channel type, termed VSOAC. Recently, Strange and Jackson (1995) have proposed that I_{Cln} could be the VSOAC channel. It is suggested that I_{Cln} is normally anchored in the cytoplasm by binding to proteins such as those described by Krapivinsky *et al.* (1994); cell swelling would release I_{Cln} from its binding sites, allowing it to be spontaneously inserted into the membrane to form a porin-like channel, thus mediating the current displayed by $I_{Clswell}$.

Our results showing that band 3 protein could act as a VSOAC but displays some functional divergences from $I_{Clswell}$ (I_{Cln}), the paradigm for VSOAC, cast doubt on the idea that VSOAC is a unique channel type. A survey of the literature also suggests that different channels may be responsible for the VSOAC-like properties. Typically, the VSOAC-mediated current is considered as outwardly rectifying and inactivated by membrane depolarisation. However (1) the degree of rectification varies widely, being large in C6 glioma cells (Jackson and Strange, 1993), slight for $I_{Clswell}$ (Ackerman *et al.* 1994) and reduced in frog erythrocytes (Hamill, 1983) and for I_{AE1} (Fiévet *et al.* 1995); and (2) the current inactivation can be very rapid and nearly complete in C6 glioma cells (Jackson and Strange, 1995), but nearly or totally nonexistent in lymphocytes (Lewis *et al.* 1993), neutrophils (Stoddard *et al.* 1993), hepatocytes (Jackson *et al.* 1996) and oocytes expressing AE1 (Fiévet *et al.* 1995). Drug sensitivity also varies between cell types: for example 1,9-dideoxyforskolin and external nucleotides, considered as typical inhibitors of VSOAC, have no effect in certain cell types (Nilius *et al.* 1994). The VSOAC-mediated conductance exhibits a broad anion selectivity and displays a high permeability (*P*) for anions over cations ($P_{cat}/P_{Cl}<0.03–0.05$). However, in skate hepatocytes, the conductance is 10 times less anion-selective ($P_{cat}/P_{Cl}<0.2–0.5$), a result consistent with kinetic studies performed in fish erythrocytes showing appreciable flux of cations through VSOAC (Bursell and Kirk, 1996; Garcia-Romeu *et al.* 1991, 1996; Joyner and Kirk, 1994; Motais *et al.* 1991; Thoroed and Fugelli, 1994). These differences do not all fall into distinct classes, but most of the extreme differences seem to occur between C6 glioma cells and epithelial cells, on the one hand, and cells from the erythroid lineage (erythrocytes, lymphocytes and neutrophils), known to possess band-3-related proteins, on the other hand.

Assuming that different types of VSOAC channels exist, it might seem reasonable that they form a series of homologous proteins, a criterion clearly not fulfilled if both I_{Cln} and AE1 function as VSOACs. The possibility that at least two such structurally different proteins show a similar transport function (i.e. the movement of structurally unrelated inorganic and organic compounds down their electrochemical gradients) is intriguing. It is also noteworthy that phospholemman, a small peptide (72 amino acids) with a single predicted transmembrane domain, also gives rise to anion-selective channels showing a high permeability to taurine when

incorporated into planar lipid bilayers (Moorman *et al.* 1995). If such a structural diversity for VSOAC exists, it could explain how the volume-sensitive amino acid efflux pathway operates in an identical manner in cell lines with or without anion exchangers (Sanchez-Olea *et al.* 1995).

Conclusion and future prospects

The trout anion exchanger AE1 appears to be a bifunctional protein, acting as an anion and amino acid channel independently of its normal counter-exchange mode. Whether the osmolyte channel is associated with one monomer or with the formation of band 3 oligomers is still unknown. Such a dual function for a membrane transport protein is probably not unique, since some other transporters have also been reported to display channel-like properties, e.g. the triose phosphate/ phosphate exchanger from the chloroplast (Schwartz *et al.* 1994) and the mitochondrial aspartate/glutamate carrier (Dierks *et al.* 1990).

The possibility that structurally diverse proteins can function as VSOACs (volume-sensitive organic osmolyte anion channels) is intriguing from a biochemical point of view. Conversely, the fact that some isoforms of AE1 (e.g. trout AE1) can function as a VSOAC whereas other very similar isoforms (e.g. mouse AE1) cannot may have important physiological as well as pathological implications. In a series of studies carried out in the last few years, Kirk and colleagues showed that the volume-activated osmolyte channel of fish erythrocytes bears a marked functional resemblance to a pathway induced in human erythrocytes after invasion by the malaria parasite *Plasmodium falciparum* (Kirk *et al.* 1994; Kirk and Horner, 1995*a*,*b*). One of the fundamental questions that remains to be addressed is whether the components of this pathway, which is crucial for the development of the parasite, are synthesised by the parasite and then inserted into the membrane or whether they are an inactive, but normal, component of the host red cell membrane and are activated by the parasite. Our finding that trout AE1 may form an anion-selective, taurine-permeable channel, whereas mouse (and human) AE1 cannot do so, strongly suggests that induction of this pathway could result from an alteration by the parasite to the human erythrocyte AE1, which then would function as a trout-like AE1.

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References

ACKERMAN, M. J., WICKMAN, K. AND CLAPHAM, D. E. (1994). Hypotonicity activates a native chloride current in *Xenopus* oocytes. *J. gen. Physiol.* **103**, 153–179.

BANDERALI, U. AND ROY, G. (1992). Anion channels for amino acids in MDCK cells. *Am. J. Physiol.* **263**, C1200–C1207.

BURSELL, J. D. H. AND KIRK, K. (1996). Swelling-activated K^+ transport *via* two functionally distinct pathways in eel erythrocytes. *Am. J. Physiol.* **270**, R61–R70.

DIERKS, T., SALENTIN, A., HEBERGER, C. AND KRAMER, R. (1990). The mitochondrial aspartate/glutamate and ADP/ATP carrier switch from obligate counterexchanger to unidirectional transport after modification by SH-reagents. *Biochim. biophys. Acta* **1028**, 268–280.

FIÉVET, B., GABILLAT, N., BORGESE, F. AND MOTAIS, R. (1995). Expression of band 3 anion exchanger induces chloride current and taurine transport: structure–function analysis. *EMBO. J.* **14**, 5158–5169.

FINCHAM, D. A., WOLOWYK, M. W. AND YOUNG, J. D. (1987). Volume-sensitive taurine transport in fish erythrocytes. *J. Membr. Biol.* **96**, 45–56.

FUGELLI, K. AND THOROED, S. M. (1986). Taurine transport associated with cell volume regulation in flounder erythrocytes under anisosmotic conditions. *J. Physiol., Lond.* **37**, 245–261.

GARCÍA, J. J., SANCHEZ-OLEA, R. AND PASANTES-MORALES, H. (1991). Taurine release associated to volume regulation in rabbit lymphocytes. *J. cell. Biochem.* **45**, 207–212.

GARCIA-ROMEU, F., BORGESE, F., GUIZOUARN, H., FIEVET, B. AND MOTAIS, R. (1996). A role for the anion exchanger AE1 (band 3 protein) in cell volume regulation. *Cell. molec. Biol.* (in press).

GARCIA-ROMEU, F., COSSINS, A. R. AND MOTAIS, R. (1991). Cell volume regulation by trout erythrocytes: characteristics of the transport systems activated by hypotonic swelling. *J. Physiol., Lond.* **440**, 547–567.

GOLDSTEIN, L. AND BRILL, S. R. (1991). Volume-activated taurine efflux from skate erythrocytes: possible band 3 involvement. *Am. J. Physiol.* **260**, R1014–R1020.

GOLDSTEIN, L. AND DAVIS, E. M. (1994). Taurine, betaine and inositol share a volume-sensitive transporter in skate erythrocyte cell membrane. *Am. J. Physiol.* **267**, R426–R431.

HAMILL, O. P. (1983). Potassium and chloride channels in red blood cells. In *Single Channel Recording* (ed. B. Sakman and E. Neher), pp. 451–471. New York: Plenum Press.

HARDY, S. P., GOODFELLOW, H. R., VALVERDE, M. A., GILL, D. R., SEPULVEDA, V. AND HIGGINS, C. F. (1995). Protein kinase C-mediated phosphorylation of the human multidrug resistance P-glycoprotein regulates cell volume-activated chloride channels. *EMBO J.* **14**, 68–75.

HAYNES, J. K. AND GOLDSTEIN, L. (1993). Volume-sensitive amino acid transport in erythrocytes of the little skate, *Raja erinacea*. *Am. J. Physiol.* **265**, R173–R179.

HIGGINS, C. F. (1995). The ABC of channel regulation. *Cell* **82**, 693–696.

HOFFMAN, E. AND SIMONSEN, L. O. (1989). Membrane mechanisms in volume and pH regulation in vertebrate cells. *Physiol. Rev.* **69**, 315–382.

JACKSON, P. S., CHURCHWELL, K., BALLATORI, N., BOYER, J. L. AND STRANGE, K. (1996). Swelling-activated anion conductance in skate hepatocytes: regulation by cell Cl and ATP. *Am. J. Physiol.* **270**, C57–C66.

JACKSON, P. S., MORRISON, R. AND STRANGE, K. (1994). The volume-sensitive organic osmolyte–anion channel VSOAC is regulated by nonhydrolytic ATP binding. *Am. J. Physiol.* **267**, C1203–C1209.

JACKSON, P. S. AND STRANGE, K. (1993). Volume-sensitive anion channels mediate swelling-activated inositol and taurine efflux. *Am. J. Physiol.* **265**, C1489–C1500.

JACKSON, P. S. AND STRANGE, K. (1995). Characterization of the

voltage-dependent properties of a volume-sensitive anion conductance. *J. gen. Physiol.* **105**, 661–676.

JOYNER, S. E. AND KIRK, K. (1994). Two pathways for choline transport in eel erythrocytes: a saturable carrier and a volume-activated channel. *Am. J. Physiol.* **267**, R773–R779.

KIRK, J. AND KIRK, K. (1994). Inhibition of volume-activated I^- and taurine efflux from HeLa cells by P-glycoprotein blockers correlates with calmodulin inhibition. *J. biol. Chem.* **269**, 29389–29394.

KIRK, K., ELLORY, J. C. AND YOUNG, J. D. (1992). Transport of organic substrates *via* a volume-activated channel. *J. biol. Chem.* **267**, 23475–23478.

KIRK, K. AND HORNER, H. A. (1995*a*). In search of a selective inhibitor of the induced transport of small solutes in *Plasmodium falciparum*-infected erythrocytes: effects of arylaminobenzoates. *Biochem. J.* **311**, 761–768.

KIRK, K. AND HORNER, H. A. (1995*b*). Novel anion dependence of induced cation transport in malaria-infected erythrocytes. *J. biol. Chem.* **270**, 24270–24275.

KIRK, K., HORNER, H. A., ELFORD, B. C., ELLORY, J. C. AND NEWBOLD, C. I. (1994). Transport of diverse substrates into malaria-infected erythrocytes *via* a pathway showing functional characteristics of a chloride channel. *J. biol. Chem.* **269**, 3339–3347.

KIRK, K. AND KIRK, J. (1993). Volume regulatory taurine release from human lung cancer cell line: Evidence for amino-acid transport *via* a volume-activated chloride channel. *FEBS Lett.***336**, 153–158.

KRAPIVINSKY, G. B., ACKERMAN, M. J., GORDON, E. A., KRAPIVINSKY, L. D. AND CLAPHAM, D. E. (1994). Molecular characterization of a swelling-induced chloride conductance regulatory protein, pI_{Cln}. *Cell* **76**, 439–448.

LAUF, P. K., BAUER, J., ADRAGNA, N. C., FUJISE, H., ZADE-OPPEN, A. M. M., RYU, K. H. AND DELPIRE, E. (1992). Erythrocyte KCl cotransport: properties and regulation. *Am. J. Physiol.* **263**, C917–C932.

LEWIS, R. A., BURSELL, J. D. H. AND KIRK, K. (1996). Anion-selectivity of the swelling-activated osmolyte channel in eel erythrocytes. *J. Membr. Biol.* **149**, 103–111.

LEWIS, R. S., ROSS, P. E. AND CAHALAN, M. D. (1993). Chloride channels activated by osmotic stress in T lymphocytes. *J. gen. Physiol.* **101**, 801–826.

MOORMAN, J. R., ACKERMAN, S. J., KOWDLEY, G. C., GRIFFIN, M. P., MOUNSEY, J. P., CHEN, Z., CALA, S. E., O'BRIAN, J. J., SZABO, G. AND JONES, L. R. (1995). Unitary anion currents through phospholemman channel molecules. *Nature* **377**, 737–740.

MOTAIS, R., FIÉVET, B., BORGESE, F. AND GARCIA-ROMEU, F. (1992). Some functional properties of band 3 protein in nucleated red cells. *Prog. Cell Res.* **2**, 253–262.

MOTAIS, R., GUIZOUARN, H. AND GARCIA-ROMEU, F. (1991). Red cell volume regulation: the pivotal role of ionic strength in controlling swelling-dependent transport systems. *Biochim. biophys. Acta* **1075**, 169–180.

MUSCH, M. W., DAVIS, E. M. AND GOLDSTEIN, L. (1994). Oligomeric forms of skate erythrocyte band 3. Effect of volume expansion. *J. biol. Chem.* **269**, 19683–19686.

NILIUS, B., SEHRER, J., VIANA, F., DE GREEF, C., RAEYMAEKERS, L., EGGERMONT, J. AND DROOGMANS, G. (1994). Volume-activated Cl currents in different mammalian non-excitable cell types. *Pflügers Arch.* **428**, 364–371.

PASSOW, H. (1986). Molecular aspects of band 3 protein-mediated anion transport across the red blood cell membrane. *Rev. Physiol. Biochem. Pharmac.* **103**, 62–186.

PAULMICHL, M., LI, Y., WICKMAN, K., ACKERMAN, M., PERALTA, E. AND CLAPHAM, D. (1992). New mammalian chloride channel identified by expression cloning. *Nature* **356**, 238–241.

ROY, G. AND MALO, C. (1992). Activation of amino acid diffusion by a volume-increase in cultured kidney (MDCK) cells. *J. Membr. Biol.* **130**, 83–90.

SANCHEZ-OLEA, R., FULLER, C., BENOS, D. AND PASANTES-MORALES, H. (1995). Volume-associated osmolyte fluxes in cell lines with or without the anion exchanger. *Am. J. Physiol.* **269**, C1280–C1286.

SANCHEZ-OLEA, R., PASANTES-MORALES, H., LAZARO, A. AND CEREIJIDO, M. (1991). Osmolarity-sensitive release of free amino acids from cultured kidney cells (MDCK) *J. Membr. Biol.* **121**, 1–9.

SARKADI, B. AND PARKER, J. C. (1991). Activation of ion transport pathways by changes in cell volume. *Biochim. biophys. Acta* **1071**, 407–427.

SCHWARZ, M., GROSS, A., STEINKAMP, T., FLUGGE, U. I. AND WAGNER, R. (1994). Ion channel properties of the reconstituted chloroplast triose phosphate/phosphate translocator. *J. biol. Chem.* **269**, 29481–29489.

SIEBENS, A. W. AND SPRING, K. R. (1989). A novel sorbitol transport mechanism in culture renal papillary epithelial cells. *Am. J. Physiol.* **257**, F937–F946.

STODDARD, J. S., STEINBACH, J. H. AND SIMCHOWITZ, L. (1993). Whole cell Cl currents in human neutrophils induced by cell swelling. *Am. J. Physiol.* **265**, C156–C165.

STRANGE, K. (1994). Are all cell volume changes the same? *News physiol. Sci.* **9**, 223–228.

STRANGE, K., EMMA, F. AND JACKSON, P. S. (1996). Cellular and molecular physiology of volume sensitive anion channels. *Am. J. Physiol.* **270**, C711–C730.

STRANGE, K. AND JACKSON, P. S. (1995). Swelling-activated organic osmolyte efflux: a new role for anion channels. *Kidney Int.* **48**, 994–1003.

STRANGE, K., MORRISON, R., SHRODE, L. AND PUTMAN, R. (1993). Mechanism and regulation of swelling-activated inositol efflux in brain glial cells. *Am. J. Physiol.* **265**, C244–C256.

STUTTS, M. J., CANESSA, C. M., OLSEN, J. C., HAMRICK, M., COHN, J. A., ROSSIER, B. C. AND BOUCHER, R. C. (1995). CFTR as a cyclic AMP-dependent regulator of sodium channels. *Science* **269**, 847–850.

THIEMANN, A., GRUNDER, S., PUSCH, M. AND JENTSCH, T. J. (1992). A chloride channel widely expressed in epithelial and non epithelial cells. *Nature* **356**, 57–60.

THOROED, S. M. AND FUGELLI, K. (1994). The Na^+-independent taurine influx in flounder erythrocytes and its association with the volume regulatory taurine efflux. *J. exp. Biol.* **186**, 245–268.

WRIGHT, C. E., TALLAN, H. H., LIN, Y. Y. AND GAULL, G. E. (1986). Taurine: biological update. *A. Rev. Biochem.* **55**, 427–453.