Eur Biophys J (2002) 31: 356–364
DOI 10.1007/s00249-002-0219-1

ARTICLE

Brett A. Cromer · Craig J. Morton · Philip G. Board
Michael W. Parker

From glutathione transferase to pore in a CLIC

Received: 21 January 2002 / Accepted: 20 February 2002 / Published online: 23 May 2002

Abstract Many plasma membrane chloride channels have been cloned and characterized in great detail. In contrast, very little is known about intracellular chloride channels. Members of a novel class of such channels, called the CLICs (chloride intracellular channels), have been identified over the last few years. A striking feature of the CLIC family of ion channels is that they can exist in a water-soluble state as well as a membrane-bound state. A major step forward in understanding the functioning of these channels has been the recent crystal structure determination of one family member, CLIC1. The structure confirms that CLICs are members of the glutathione S-transferase superfamily and provides clues as to how CLICs can insert into membranes to form chloride channels.

Keywords Chloride channels · Crystal structures · CLICs · Glutathione transferases · Pore-forming toxins

Introduction

Chloride ion channels are found on the plasma membrane of virtually all eukaryotic cells as well as in various intracellular organelles such as endosomes and the golgi complex. They are involved in a diverse range of physiological processes, including response to certain neurotransmitters, bone resorption, transepithelial transport, acidification of intracellular compartments, regulation of potential across membranes, cell volume homeostasis and control of secretion and absorption of salt by cells (Strange et al. 1996). The major classes of chloride channels are the ligand-gated ion channels (GABA and glycine receptors), cystic fibrosis transmembrane conductance regulators (CTFR), chloride channels (ClCs) and chloride intracellular channels (CLICs) (al-Awqati 1995; Jentsch and Gunther 1997). Malfunctioning of chloride channels can lead to serious diseases such as several myotonias (Koch et al. 1992), Dent's disease (Lloyd et al. 1996), Bartter's syndrome (Simon et al. 1997) and cystic fibrosis (Riordan et al. 1989).

CLICs are the most recently discovered family of chloride ion channels. They have a broad tissue distribution. As well as being found in plasma membranes they have also been located in organelle membranes such as mitochondria (Fernandez-Salas et al. 1999), nuclear membranes (Valenzuela et al. 1997), large dense core vesicles (Chuang et al. 1999), trans-golgi vesicles (Edwards 1999), secretory vesicles (Redhead et al. 1997) and the endoplasmic reticular membrane (Duncan et al. 1997). CLIC channels contribute to a variety of important physiological processes such as cell division (Valenzuela et al. 2000), kidney function (Landry et al. 1989) and facilitating the acidification of the ruffled border in bone resorption (Schlesinger et al. 1997a). One CLIC, parchorin (so-called because of its enrichment in parietal cells and choroid plexus), is translocated to the plasma membrane on chloride ion depletion of the extracellular medium of water secreting cells (Nishizawa et al. 2000). Several CLICs interact with protein kinases and hence may play a role in signal transduction events (Qian et al. 1999; Edwards and Kapadia 2000; Nishizawa et al. 2000).

So far, seven members of the CLIC family have been identified: CLIC1 (also called nuclear chloride channel-27 or NCC27) (Valenzuela et al. 1997), CLIC2 (Heiss and Poustka 1997), CLIC3 (Qian et al. 1999), CLIC4 (Duncan et al. 1997), CLIC5 (Berryman and Bretscher

Presented at the Australian Biophysical Society Meeting, 2001

B.A. Cromer · C.J. Morton · M.W. Parker (✉)
Biota Structural Biology Laboratory,
St. Vincent's Institute of Medical Research,
9 Princes Street, Fitzroy, Victoria 3065, Australia
E-mail: mwp@rubens.its.unimelb.edu.au
Fax: +61-3-94162676

P.G. Board
John Curtin School of Medical Research,
Australian National University, PO Box 334,
Canberra, ACT 2601, Australia

2000), p64 (Landry et al. 1993) and parchorin (Nishizawa et al. 2000). It appears p64 is a splice variant of CLIC5, based on an analysis of human genome sequence databases (B.A. Cromer, unpublished observations). The first five members are approximately 240 amino acid residues long, whereas p64 and parchorin have long N-terminal extensions. Members of the CLIC family are highly conserved: CLICs share between 47% and 76% pairwise sequence identity with each other (Fig. 1).

Until very recently it was not clear whether CLICs modulate channels or actually form channels on their own. Since CLICs are largely intracellular and lack well-defined membrane-spanning domains in their primary structure, they were thought more likely to function as regulators or activators of chloride channels. However, recent data suggest they can indeed form ion channels. CLIC1 has been shown to insert into phospholipid vesicles or artificial lipid bilayers to form channels with similar properties to those observed in vivo, including a selectivity for chloride ions (Tulk et al. 2000; Harrop et al. 2001). These studies do not reveal how CLICs might insert into membranes, although they do indicate that CLICs do not need a receptor for insertion. The CLICs possess two regions of significant hydrophobicity

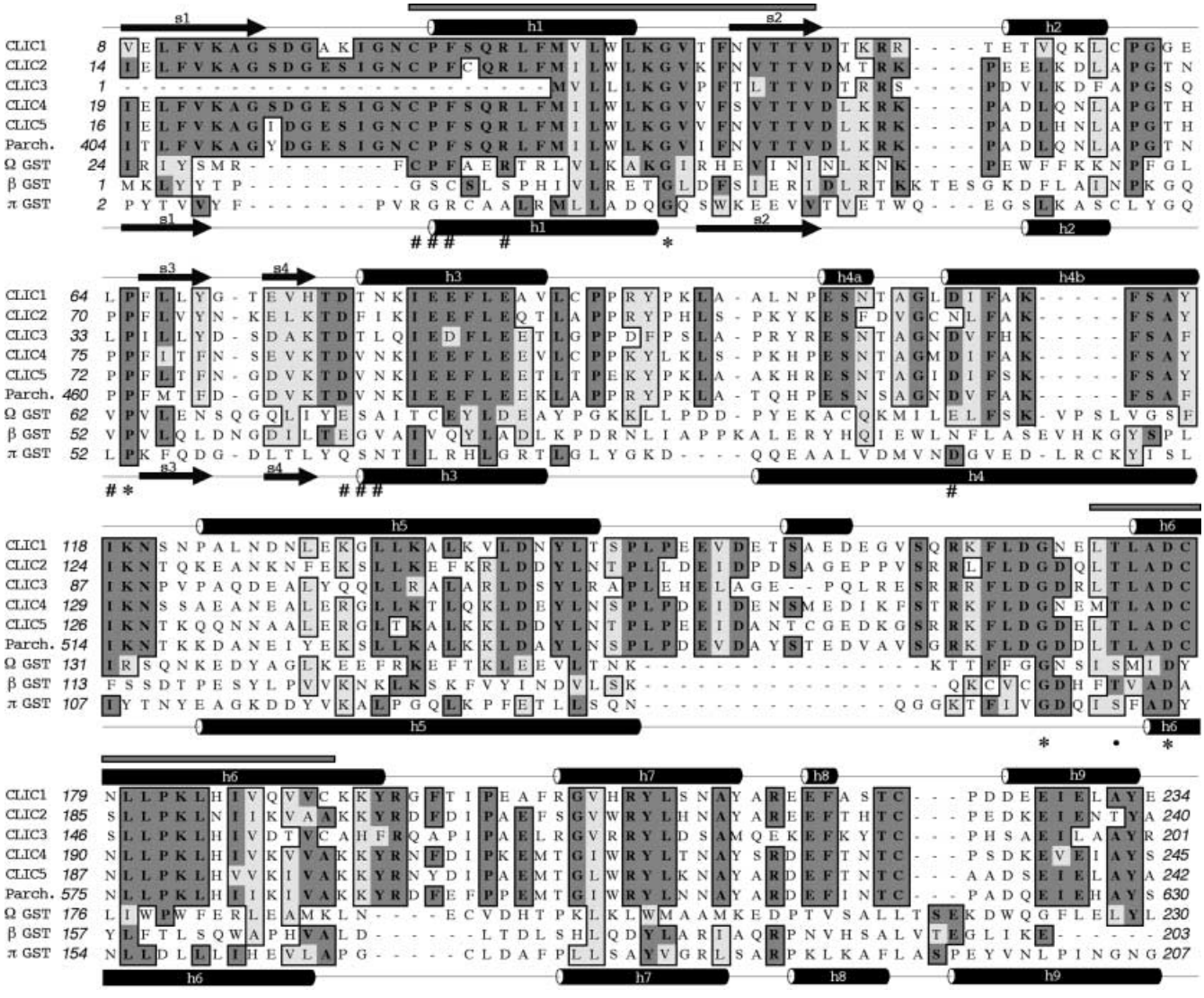


Fig. 1. Comparison of CLICs and GSTs. The secondary structure (*cylinders* represent helices and *arrows* represent strands) of CLIC1 is shown above the alignment and omega class GST secondary structure below the alignment. Conserved residues are *highlighted in boxes*, with strictly conserved residues in *dark shade* and similar residues in *lighter shade*. Invariant residues from both classes are designated by *asterisks*. A *black dot* denotes a conserved Ser/Thr that takes part in a helix-capping motif at the N-terminal end of helix α6. Residues involved in GSH binding are denoted by a *hash*. Putative transmembrane regions are also highlighted by the *striped bars* above each region. Database accession numbers for the sequences used in the alignment are taken from SwissProt unless otherwise designated as GB (Genbank): human CLIC1, O00299; human CLIC2, O15247; human CLIC3, O95833; human CLIC4, Q9Y696; human CLIC5, AAF66928 (GB); rabbit parchorin, BAA94345 (GB); human omega GST, P78417; *Proteus mirabilis* beta GST, P15214; human pi GST, P09211. The alignment was produced using Clustal W (Thompson et al. 1994) and modified to fit alignments based on the available crystal structures

in their primary structure, so one or both of these regions may be involved in the insertion process (Berryman and Bretscher 2000). Proteolytic and epitope mapping studies of CLICs suggest they insert into membranes with the N-terminus travelling across the membrane whereas the C-terminus remains on the cytoplasmic side (Duncan et al. 1997; Tonini et al. 2000).

A recent search of sequence data bases revealed a link between the CLIC family and the glutathione S-transferase (GST) superfamily (Fig. 1) (Dulhunty et al. 2000). Although the sequence identity was low (approximately 15% with an omega class GST), the CLIC1 sequence was clearly compatible with the canonical GST fold, based on threading results. Four key residues were identified that are conserved in virtually all GSTs and CLICs: a *cis*-proline residue that provides the correct active site geometry for binding glutathione (GSH) in GSTs, an aspartic acid residue that forms an N-terminal helix capping interaction and two glycine residues that play structural roles (Fig. 1). In addition, a Cys-Pro-Phe motif, which includes the active site cysteine residue of the omega class GST, is also found in the CLICs. Another link was the observation that a chloride channel inhibitor, indanyloxyacetic acid-94, which inhibits CLIC1 channels, is a homologue of the well-known GST inhibitor ethacrynic acid (Tulk et al. 2000; Harrop et al. 2001). Finally, both families have known associations with protein kinases (Alder et al. 1999; Qian et al. 1999; Edwards and Kapadia 2000; Nishizawa et al. 2000; Cho et al. 2001).

CLIC structure

The first crystal structure of a CLIC family member, CLIC1, has been determined only recently (Harrop et al. 2001). The structure presented, determined at 1.4 Å resolution, is of the soluble form of the channel protein (Fig. 2). In addition, the structure of the GSH complex was determined at 1.8 Å resolution. CLIC1 is monomeric and folds into two domains: an N-terminal domain possessing a thioredoxin fold and an all-helical C-terminal domain. This work confirmed that CLICs adopt the canonical three-dimensional fold of the GST superfamily as predicted by Dulhunty and co-workers (2000), despite sequence identities of less than 15% between the two families.

Harrop et al. (2001) have argued that the active site of CLIC1 resembles glutaredoxin, a member of the thioredoxin superfamily from which GSTs have evolved. Like glutaredoxin (and some GSTs), CLIC1 has a redox-active cysteine residue near the N-terminus which can form a mixed disulfide with GSH. Interestingly, CLIC2 possesses a second cysteine, three residues away, and hence has the thioredoxin active site motif of Cys-X-X-Cys. Thus CLICs might function as thioredoxins or glutaredoxins. By analogy with thioredoxins, Harrop et al. (2001) have postulated that the wide active site of CLIC1 might accommodate a putative protein target.

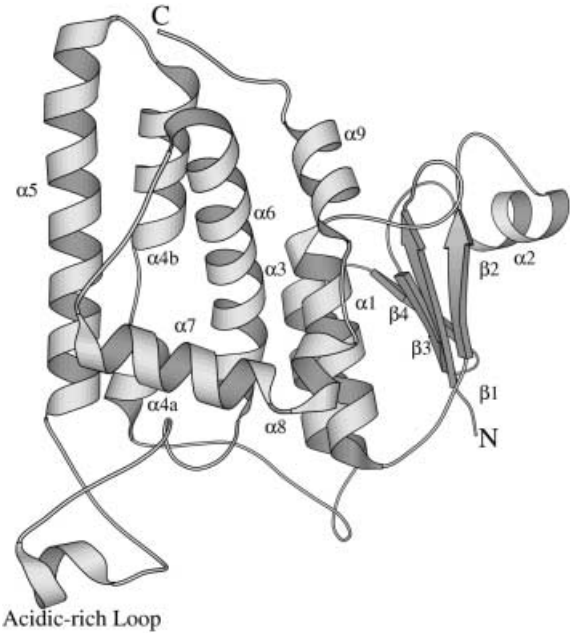


Fig. 2. Three-dimensional structure of CLIC1 (Harrop et al. 2001). The locations of secondary structure and the acidic-rich loop are *highlighted*. This figure was produced using MOLSCRIPT (Kraulis 1991)

Functional diversity of the GST superfamily

The enzyme superfamily of GSTs is a vital component of the cellular detoxification of a large variety of endogenous and exogenous toxins (Sheehan et al. 2001). They detoxify the toxins by conjugating them with GSH, the first step in the breakdown and eventual excretion of the toxins from the organism. They are widely distributed, being found in most tissues in the body and are ubiquitous in aerobic organisms. The enzyme's ability to recognize a diverse range of substrates is due, in part, to the existence of isoforms. Soluble GSTs can be grouped into at least 12 classes called alpha, beta, delta, mu, phi, pi, theta, kappa, sigma, tau, omega and zeta (Sheehan et al. 2001 and refs. therein). The different classes are highly divergent, with sequence identities typically between 10% and 30%.

Although GSTs are well known for their detoxification activity by catalysing GSH conjugation to many exogenous and endogenous compounds, they have many other possible functions and activities. These include storage and transport of compounds in the liver (Litwack et al. 1971), peroxidase activity (Mannervik and Danielson 1988), structural roles such as in S-crystallins (Mannervik and Danielson 1988), dehalogenation reactions (McCarthy et al. 1996), prostaglandin synthesis (Kanaoka et al. 1997), isomerase activity (Fernández-Cañón and Peñalva 1998), regulation of stress kinases (Alder et al. 1999; Cho et al. 2001), cell protection from radiation damage (Kodym et al. 1999), a target for "tissue" transglutaminase in neural cells committed to apoptosis (Piredda et al. 1999), thiol transferase activity

(Board et al. 2000), dehydroascorbate reduction (Board et al. 2000), inhibition of the proapoptotic action of Bax (Kampranis et al. 2000), modulation of calcium channels (Dulhunty et al. 2000) and an NO carrier (Lo Bello et al. 2001). Thus the finding that some GSTs may function as chloride channels in the form of CLICs may not come as a big surprise, given their previously known functional diversity.

Structural studies of GSTs

Representative crystal structures of GSTs from nearly every class are available (see Sheehan et al. 2001 for a review). These studies have shown that the overall polypeptide fold is very similar: the N-terminal domain consists of a central core of a mixed four-stranded β-sheet surrounded on one side by a pair of α-helices and on the other by another helix (helix $\alpha 2$) and irregular structure, whereas the C-terminal domain is all α-helical (Fig. 3). Each class exhibits unique features, especially around the active site and the C-terminus. The active site consists of a GSH binding site (G-site) and a binding site for hydrophobic electrophilic compounds (the H-site). A hallmark feature of many GSTs is the presence of a catalytic hydroxylated residue (Tyr or Ser) near the N-terminus that stabilizes the thiolate form of GSH by contributing a hydrogen bond to it. Another characteristic feature of GSTs is the mode of GSH binding: GSH binds in an extended fashion so that it forms an antiparallel β-sheet-like interaction between its peptide backbone and the backbone of the residue preceding the strictly conserved *cis*-proline residue in the protein. GSTs are normally homodimeric, although there are examples of heterodimers and at least two cases of a monomer (McCarthy et al. 1996; Polekhina et al. 2001).

Evolutionary development of GSTs

The close three-dimensional structural similarity between the N-terminal domains of GSTs and the thioredoxin fold was noted in the analysis of the first GST crystal structure (Reinemer et al. 1991). However, there was no sequence similarity between the two enzymes beyond the structurally important *cis*-proline residue found in the active sites of both. The subsequent structure determinations of the delta (Wilce et al. 1995) and beta classes (Rossjohn et al. 1998b) revealed sequence relationships between the thioredoxin and GST superfamilies, supporting the proposal that GSTs evolved from a thioredoxin ancestor. The structure of the beta class enzyme, a GST that is widely distributed in bacteria, exhibited two features that were unique amongst the GST superfamily and which were considered relics of the monomeric thioredoxin-like molecule from which GSTs likely evolved. Firstly, the subunit and domain interfaces were quite polar, unlike most GSTs where these interfaces are normally quite hydrophobic. Secondly, a cysteine residue formed a mixed disulfide with GSH, in place of the usual catalytic Tyr or Ser residue seen in the more evolved GST classes. The cysteine superimposed closely with one of the catalytic cysteine residues in the thioredoxin superfamily. Recently a crystal structure of an omega class GST was determined and, like the beta class enzyme, the protein was shown to have a cysteine residue instead of the catalytic Tyr or Ser in the active site which also formed a mixed disulfide bridge with GSH (Board et al. 2000). These observations have led to suggestions that the beta and omega class enzymes may possess redox activity (Rossjohn et al. 1998b; Zakharyan et al. 2001). To date, the evolutionary history of the GST C-terminal domain has remained a mystery.

CLICs as GSTs

The question arises as to whether CLICs simply fold like GSTs (convergent evolution) or whether they are related by a common ancestor (divergent evolution). An analysis of the CLIC structure provides some clues. CLICs adopt the same topology of secondary structure as GSTs and superpositions reveal that the CLIC and GST folds are closely related (Harrop et al. 2001). CLIC1 most closely resembles beta and omega GSTs with root-mean-square (r.m.s.) deviations on α-carbon atoms of approximately 1.6 Å against each GST. This value is well within the range found for superposition of GSTs from different classes. The CLIC1 domain interface is predominantly hydrophobic and the linker region between the two domains provides a large hydrophobic residue (Leu96 in CLIC1) as a wedge between the two domains, as found in most GST structures. The partly buried H-site in CLIC1 is reminiscent of the human alpha (Sinning et al. 1993) and theta class structures (Rossjohn et al. 1998a), where the active sites are also partly covered by a C-terminal helical extension. Nevertheless, a superposition of the model of pi class GST complexed to ethacrynic acid (Oakley et al. 1997b) onto the CLIC1 structure shows that there is sufficient space in the H-site of CLIC1 for the inhibitor. Thus it seems likely that the CLIC inhibitor indanyloxyacetic acid-94, a homologue of ethacrynic acid, would also bind in the H-site in similar fashion as seen for ethacrynic acid in pi class GST. Helix $\alpha 2$ is normally a highly flexible region in mammalian GSTs but is found anchored to the β-sheet core of the N-terminal domain, via an aromatic residue nestling into a hydrophobic pocket, in non-mammalian GSTs (Fig. 3) (Wilce et al. 1995; Reinemer et al. 1996; Rossjohn et al. 1998b). In CLIC1 this helix is clearly anchored to the core via Leu58 of the helix which packs into a hydrophobic pocket consisting of Phe11, Phe66, Leu68 and Val73 (Fig. 2).

The most striking feature of the CLIC1 active site is the presence of a mixed disulfide bridge between Cys24 and GSH (Fig. 4). This feature is reminiscent of the

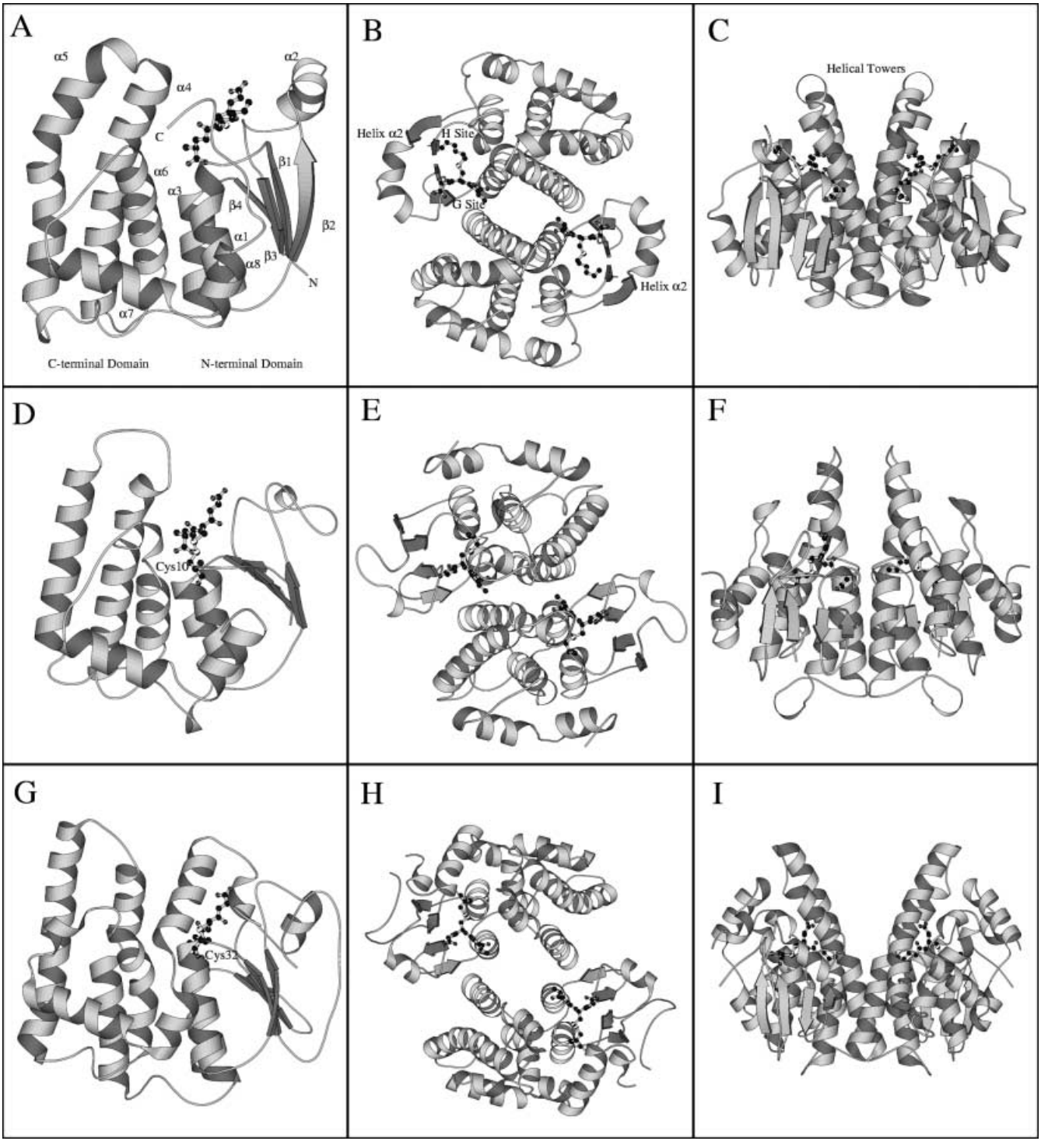


Fig. 3A–I. The common topology of the GST superfamily. In each row the fold of the monomer and two orthogonal views of the dimer for each GST are shown. Protein data bank codes and references for the structures are: **A**–**C**, pi class, 9GSS (Oakley et al. 1997a); **D**–**F**, beta class, 1PMT (Rossjohn et al. 1998b); **G**–**I**, omega class, 1EEM (Board et al. 2000). The pi class GST can be considered the archetypal GST: key GST features are shown in the pi class figures with the inhibitor S-hexyl GSH shown in panels **A**, **B** and **C** in order to highlight the H-site. The disulfide-bound GSH in the beta and omega classes is also highlighted. This figure was produced using MOLSCRIPT (Kraulis 1991)

mixed disulfides found in the beta (Rossjohn et al. 1998b) and omega class (Board et al. 2000) GSTs. Harrop et al. (2001) noted there were are no protein interactions with N-terminal amino group of the γ-glutamyl moiety or the main-chain carbonyl group of the cysteinyl moiety of GSH. It is possible that physiologically relevant GSH binding requires CLIC1 dimerization since in many GSTs an acidic residue from the neighbouring monomer forms a salt bridge with the

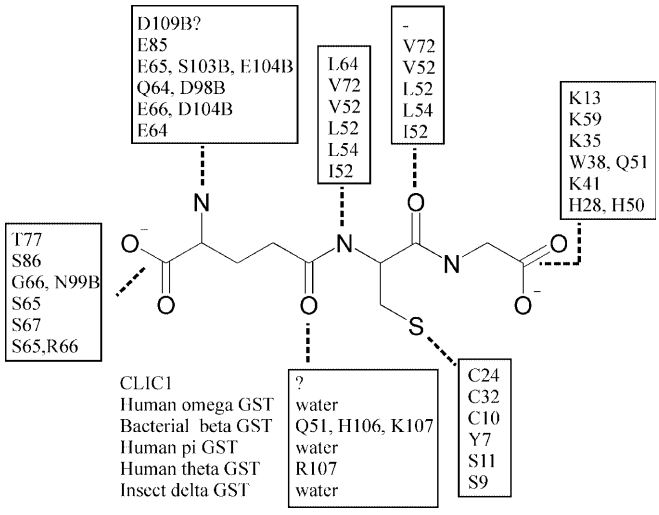


Fig. 4. Schematic illustrating the residues contacting GSH in CLIC1 and certain classes of GSTs

N-terminal amino group of the γ-glutamyl moiety of GSH. Indeed, there is an aspartic acid residue in CLIC1 at the equivalent position (residue 109) that could fulfil this role (Fig. 1). This residue is conserved in all members of the CLIC family except CLIC2, where it is an asparagine. Otherwise the CLIC1 residues involved in binding GSH are nearly all conserved or conservatively substituted in the GSTs (Fig. 4). Another significant feature of the CLIC1 active site is that it is located in a wide crevice which is reminiscent of some GST active sites, particularly the omega class GST where it has been suggested that the site is sufficiently large to accept protein targets (Board et al. 2000).

An obvious difference between CLIC1 and GSTs is that the former is a monomer whereas the latter are dimers. However, there are at least two reported cases of monomeric GSTs (McCarthy et al. 1996; Polekhina et al. 2001) and in the beta (Rossjohn et al. 1998b) and sigma class (Ji et al. 1995) GSTs the dimer interface is very polar, which is thought to be a relic of their ancestral origins within the monomeric thioredoxin superfamily.

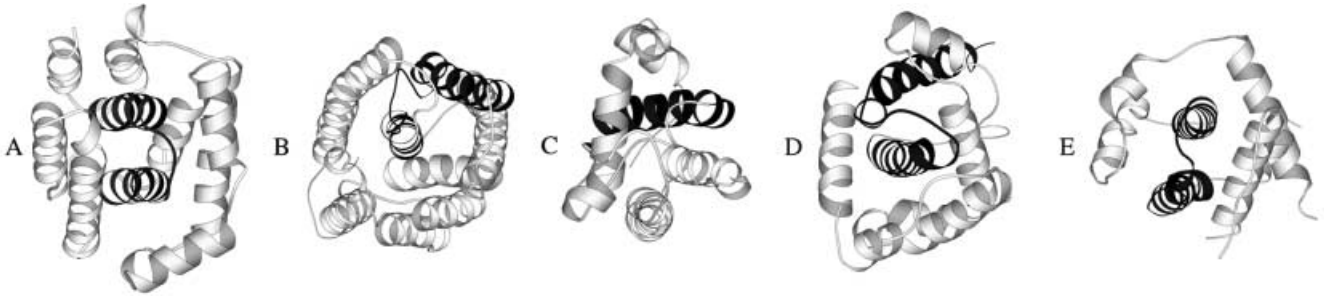
Another obvious difference between the two families is the long, highly charged loop region (from Pro147 to Gln164) at the base of the CLIC molecule (cf. Figs. 2 and 3). In CLIC1 there are seven acidic residues in this region and the number varies between three and six in other family members. Another hotspot of negative charge occurs in the C-terminal helix, where there are six acidic residues. It is suggested below that these features may play a key role in membrane insertion of CLICs.

How do CLICs form channels in membranes?

To date, no other GSTs have been shown to form channels, although omega class GSTs have been shown to modulate ryanodine receptors which are calcium channels located in the endoplasmic reticulum of various cells (Dulhunty et al. 2000). Of possible relevance to CLICs, the channel modulating activity was dependent on the GST being active and the active site cysteine was shown to be essential.

There are two regions of significant hydrophobicity in CLICs, as judged by hydrophobicity plots. One region is located in the N-terminal domain and comprises helix 1 and β-strand 2 (Figs. 1 and 2) (Landry et al. 1993; Berryman and Bretscher 2000; Harrop et al. 2001). The second region is located in the C-terminal domain and comprises most of helix 6 and part of the loop preceding it (Figs. 1 and 2) (Landry et al.1993; Qian et al. 1999; Berryman and Bretscher 2000). However, both regions are mostly hydrophobic in all GSTs, as they form the central core of the protein and provide critical interactions in keeping the two domains of the monomer together (Figs. 1, 3). Since the N-terminal domain has evolved from the thioredoxin superfamily, it seems unlikely that it will unfold readily to form a membrane-interacting domain. On the other hand, the C-terminal domain has no known evolutionary origin, so this domain may possess pore-forming activity. The topology of the C-terminal domain is not too dissimilar to that found in a number of pore-forming proteins (Fig. 5).

Fig. 5A–E. Ribbon representations of the protein insertion/translocation domains of various pore-forming proteins. The helices thought to form transmembrane domains in the channel form of each protein are shown in *dark shade*. By analogy with the other proteins, it is likely that CLIC would have a second helix involved in channel formation. **A** The pore-forming domain of colicin A (Parker et al. 1989). **B** The pore-forming domain of insecticidal δ-endotoxin (Li et al. 1991). **C** The C-terminal domain of CLIC1 (Harrop et al. 2001). **D** The translocation domain of diphtheria toxin (Choe et al. 1992). **E** The anti-apoptotic protein Bcl-x_L (Muchmore et al. 1996). All figures were produced by the computer program MOLSCRIPT (Kraulis 1991)



Pore-forming toxins and CLICs

The first crystal structure of a pore-forming protein, colicin A, revealed that its pore-forming domain consisted of a bundle of eight α-helices surrounding two hydrophobic helices that were completely buried within the protein (Fig. 5A) (Parker et al. 1989). This hydrophobic helical hairpin was hypothesized to lead insertion into membranes and to form the transmembrane channel. This idea led to the ''umbrella'' hypothesis of membrane insertion whereby the outer helical layers peel away from the central hairpin like the opening up of an umbrella (Parker and Pattus 1993). Subsequent experiments have supported the hypothesis (Lambotte et al. 1998 and refs. therein). Colicin A was also shown to form a molten globule state at low pH, corresponding to the pH levels found at the surface of biological membranes (van der Goot et al. 1991). A molten globule state would reduce the energy barrier required in unmasking the hydrophobic hairpin for membrane insertion. The second structure of a pore-forming toxin, that of insecticidal δ-endotoxin, revealed a pore-forming domain with similarities to the colicin fold (Li et al. 1991). In this toxin the domain consists of a seven-helix bundle with a central helix completely surrounded by the six outer helices (Fig. 5B). The structure of diphtheria toxin revealed a translocation domain consisting of nine helices arranged in three layers, with two helices forming a hydrophobic hairpin (Fig. 5D) (Choe et al. 1992). An interesting feature of the structure was the presence of six acidic residues on loops located on one face of the helical barrel. It was proposed that low pH (as found in the endosomal compartment that the toxin uses to enter the cell) would neutralize these loops, rendering the face more hydrophobic and hence the lowering the energy costs of inserting the helical hairpin into the membrane. More recently, members of the Bcl-2 family of apoptotic proteins have been shown to form ion channels in similar fashion to these pore-forming proteins. The three-dimensional structure of Bcl-x_L showed that its fold was reminiscent of the pore-forming/translocation domains of the above-mentioned toxins (Fig. 5E) (Muchmore et al. 1996). This discovery led to the hypothesis that Bcl-x_L might also form channels and this was subsequently proven (Minn et al. 1997). Deletion mutant studies are consistent with the central pair of primarily hydrophobic helices being directly involved in channel formation (Schendel et al. 1997). Other members of the family, such as Bcl-2 (Schendel et al. 1997), Bax (Antonsson et al. 1997; Schlesinger et al. 1997b) and Bid (Schendel et al. 1999), have also been shown to form channels.

None of the pore-forming proteins discussed above have any detectable sequence similarity between them. Nevertheless, they adopt similar folds consisting of a bundle of alpha helices organized in a layered structure, with some helices completely buried. In all cases, at least one helix of sufficient length to span a membrane could be identified. Low pH is a requirement for membrane insertion of three of the proteins: colicins, diphtheria toxin and Bcl-x_L (Minn et al. 1997). In these cases, low pH appears to play a role in promoting membrane insertion by either inducing a molten globule state and/or in making a part of the protein more hydrophobic by neutralizing acidic residues. The C-terminal domain of CLICs appears to bear some resemblance to the pore-forming domains of the above-mentioned proteins (Fig. 5C). These include an α-helical bundle with a 30-Å long hydrophobic helix (helix α6) and two regions of significant negative charge. The presence of the highly negatively charged loop region (from Pro147 to Gln164) at the base of CLIC1 and the acidic helix α9 (Fig. 2) is reminiscent of the highly negatively charged loop regions of diphtheria toxin. This leads to the speculation that protonation of these residues, due to the low pH at the surface of a biological membrane, would cause the loop to become very hydrophobic and in turn cause the protein to partition into the membrane.

Although the similarities between C-terminal domains of CLICs and the pore-forming domains of bacterial toxins are intriguing, other scenarios cannot be discarded. For example, some bacterial toxins such as *Staphylococcus aureus* α-hemolysin (Song et al. 1996; Olson et al. 1999), anthrax protective antigen (Petosa et al. 1997) and perfringolysin O (Rossjohn et al. 1997) exhibit significant changes in secondary structure in going from the water-soluble to the membrane channel state. In the case of perfringolysin, a series of six helices are converted to a couple of beta hairpins in going from one state to the other (Shatursky et al. 1999). Hence the structure of the water-soluble state of CLIC1 may not be providing any clues as to the structure of the membrane-bound state!

Concluding remarks

The finding that CLICs are members of the GST superfamily have led to the hypothesis in this paper that the CLIC/GST C-terminal domain may possess pore-forming activity because of structural similarities with certain pore-forming toxins. It is possible that the ancestral protein from which the C-terminal domain evolved possessed this activity, although it is equally possible that the activity was gained during evolution. It should be stressed that it is highly unlikely that all GSTs have pore-forming activity, since their primary role appears to be cellular detoxification and any ancestral pore-forming activity has likely been lost as they evolved their primary function.

Acknowledgements We thank Dr. Paul Curmi for providing his model of CLIC1 prior to public release. M.W.P. would like to thanks all present and past members of his laboratory and his collaborators who have made major contributions to the structural studies of toxins and GSTs discussed in this paper. This work was supported in part by grants from the Australian Research Council and National Health and Medical Research Council of Australia. M.W.P. is an Australian Research Council Senior Research Fellow.

References

al-Awqati Q (1995) Chloride channels of intracellular organelles. Curr Opin Cell Biol 7:504–508

Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, Pincus MR, Sardana M, Henderson CJ, Wolf CR, Davis RJ, Ronai Z (1999) Regulation of JNK signaling by GSTp. EMBO J 18:1321–1334

Antonsson B, Conti F, Ciavatta A, Montessuit S, Lewis S, Martinou I, Bernasconi L, Bernard A, Mermod JJ, Mazzei G, Maundrell K, Gambale , Sadoul R, Martinou JC (1997) Inhibition of Bax channel-forming activity by Bcl-2. Science 277:370–372

Berryman M, Bretscher A (2000) Identification of a novel member of the chloride intracellular channel gene family (CLIC5) that associates with the actin cytoskeleton of placental microvilli. Mol Biol Cell 11:1509–1521

Board PG, Coggan M, Chelvanayagam G, Easteal S, Jermiin LS, Schulte GK, Danley DE, Hoth LR, Griffor MC, Kamath AV, Rosner MH, Chrunyk BA, Perregaux DE, Gabel CA, Geoghegan KF, Pandit J (2000) Identification, characterization, and crystal structure of the omega class glutathione transferases. J Biol Chem 275:24798–24806

Cho SG, Lee,YH, Park HS, Ryoo K, Kang KW, Park J, Eom SJ, Kim MJ, Chang TS, Choi SY, Shim J, Kim Y, Dong MS, Lee MJ, Kim SG, Ichijo H, Choi EJ (2001) Glutathione S-transferase mu modulates the stress-activated signals by suppressing apoptosis signal-regulating kinase 1. J Biol Chem 276:12749–12755

Choe S, Bennett MJ, Fujii G, Curmi PM, Kantardjieff KA, Collier RJ, Eisenberg D (1992) The crystal structure of diphtheria toxin. Nature 357:216–222

Chuang JZ, Milner TA, Zhu M, Sung CH (1999) A 29 kDa intracellular chloride channel p64H1 is associated with large dense core vesicles in rat hippocampal neurons. J Neurosci 19:2919–2928

Dulhunty A, Gage P, Curtis S, Chelvanayagam G, Board P (2000) The glutathione transferase structural family includes a nuclear chloride channel and a ryanodine receptor calcium release channel modulator. J Biol Chem 276:3319–3323

Duncan RR, Westwood PK, Boyd A, Ashley RH (1997) Rat brain p64H1: expression of a new member of the p64 chloride channel protein family in endoplasmic reticulum. J Biol Chem 272:23880–23886

Edwards JC (1999) A novel p64-related Cl^- channel: subcellular distribution and nephron segment-specific expression. Am J Physiol 276:F398–F408

Edwards JC, Kapadia S (2000) Regulation of the bovine kidney microsomal chloride channel p64 by p59fyn, a src family kinase. J Biol Chem 275:31826–31832

Fernández-Cañón JM, Peñalva MA (1998) Characterization of a fungal maleylacetoacetate isomerase gene and identification of its human homologue. J Biol Chem 273:329–337

Fernandez-Salas E, Sagar M, Cheng C, Yuspa SH, Weinberg WC (1999) p53 and tumor necrosis factor α regulate the expression of a mitochondrial chloride channel protein. J Biol Chem 274:36488–36497

Harrop SJ, DeMaere MZ, Fairlie WD, Reztsova T, Valenzuela SM, Mazzanti M, Tonini, R, Qui MR, Jankova L, Warton K, Bauskin AR, Wu WM, Pankhurst S, Campbell TJ, Breit SN, Curmi PMG (2001) Crystal structure of the soluble form of the intracellular chloride ion channel CLIC1 (NCC27) at 1.4 Å resolution. J Biol Chem 276:44993–45000

Heiss NS, Poustka A (1997) Genomic structure of a novel chloride channel gene, CLIC2, in xq28. Genomics 45:224–228

Jentsch TJ, Gunther W (1997) Chloride channels: an emerging molecular picture. Bioessays 19:117–126

Ji X, van Rosenvinge EC, Johnson WW, Tomarev SI, Piatigorsky J, Armstrong RN, Gilliland GL (1995) Three-dimensional structure, catalytic properties, and evolution of a sigma class glutathione transferase from squid, a progenitor of the lens S-crystallins of cephalopods. Biochemistry 34:5317–5328

Kampranis SC, Damianova R, Atallah M, Toby G, Kondi G, Tsichlis P, Makris AM (2000) A novel plant glutathione S-transferase/peroxidase suppresses Bax lethality in yeast. J Biol Chem 275:29207–29216

Kanaoka Y, Ago H, Inagaki E, Nanayama T, Miyano M, Kikuno R, Fujii Y, Eguchi N, Toh H, Urade Y, Hayaishi O (1997) Cloning and crystal structure of hematopoietic prostaglandin D synthase. Cell 90:1085–1095

Koch MC, Steinmeyer K, Lorenz C, Ricker K, Wolf F, Otto M, Zoll B, Lehmann-Horn F, Grzeschik KH, Jentsch TJ (1992) The skeletal muscle chloride channel in dominant and recessive human myotonia. Science 257:797–800

Kodym R, Calkins P, Story M (1999) The cloning and characterization of a new stress response protein. A mammalian member of a family of theta class glutathione S-transferase-like proteins. J Biol Chem 274:5131–5137

Kraulis P (1991) MOLSCRIPT: a program to produce both detailed and schematic plots of proteins. J Appl Crystallog 24:946–950

Lambotte S, Jasperse P, Bechinger B (1998) Orientational distribution of alpha-helices in the colicin B and E1 channel domains: a one and two dimensional ^{15}N solid-state NMR investigation in uniaxially aligned phospholipid bilayers. Biochemistry 37:16–22

Landry DW, Akabas MH, Redhead CR, Edelman A, Cragoe EJ Jr, al-Awqati Q (1989) Purification and reconstitution of chloride channels from kidney and trachea. Science 244:1469–1472

Landry D, Sullivan S, Nicolaides M, Redhead C, Edelman A, Field, M, al-Awqati Q, Edwards J (1993) Molecular cloning and characterization of p64, a chloride channel from kidney microsomes. J Biol Chem 268:14948–14955

Li J, Carrol J, Ellar DJ (1991) Crystal structure of insecticidal delta-endotoxin from *Bacillus thuringiensis* at 2.5 Å resolution. Nature 353:815–821

Litwack G, Ketterer B, Arias IM (1971) Ligandin: a hepatic protein which binds steroids, bilirubin, carcinogens and a number of exogenous organic anions. Nature 234:466–467

Lloyd SE, Pearce SH, Fisher SE, Steinmeyer K, Schwappach B, Scheinman SJ, Harding B, Bolino A, Devoto M, Goodyer P, Rigden SP, Wrong O, Jentsch TJ, Craig IW, Thakker RV (1996) A common molecular basis for three inherited kidney stone diseases. Nature 379:445–449

Lo Bello M, Nuccetelli M, Caccuri AM, Stella L, Parker MW, Rossjohn J, McKinstry WJ, Federici G, Polizio F, Pedersen JZ, Ricci G (2001) Human glutathione transferase P1-1 and nitric oxide carriers: a new role for an old enzyme. J Biol Chem 276:42138–42145

Mannervik B, Danielson UH(1988) Glutathione transferases – structure and catalytic activity. CRC Crit Rev Biochem 23:283–337

McCarthy DL, Navarrete S, Willett WS, Babbitt PC, Copley SD (1996) Exploration of the relationship between tetrachlorohydroquinone dehalogenase and the glutathione S-transferase superfamily. Biochemistry 35:14634–14642

Minn AJ, Velez P, Schendel SL, Liang H, Muchmore SW, Fesik SW, Fill M, Thompson CB (1997) Bcl-x(L) forms an ion channel in synthetic lipid membranes. Nature 385:353–357

Muchmore SW, Sattler M, Liang H, Meadows RP, Harlan JE, Yoon HS, Nettesheim D, Chang BS, Thompson CB, Wong SL, Ng SL, Fesik SW (1996) X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. Nature 381:335–341

Nishizawa T, Nagao T, Iwatsubo T, Forte JG, Urushidani T (2000) Molecular cloning and characterization of a novel chloride intracellular channel-related protein, parchorin, expressed in water-secreting cells. J Biol Chem 275:11164–11173

Oakley AJ, Lo Bello M, Battistoni A, Ricci G, Rossjohn J, Villar HO, Parker MW (1997a) The structures of human glutathione transferase P1-1 in complex with glutathione and various inhibitors at high resolution. J Mol Biol 274:84–100

Oakley AJ, Rossjohn J, Lo Bello M, Caccuri AM, Federici G, Parker MW (1997b) The three-dimensional structure of the

human pi class glutathione transferase P1-1 in complex with the inhibitor ethacrynic acid and its glutathione conjugate. Biochemistry 36:576–585

Olson R, Nariya H, Yokota K, Kamio Y, Gouaux E (1999) Crystal structure of staphylococcal LukF delineates conformational changes accompanying formation of a transmembrane channel. Nat Struct Biol 6:134–140

Parker MW, Pattus F (1993) A common packaging motif in bacterial protein toxins. Trends Biochem Sci 18:391–395

Parker MW, Pattus F, Tucker AD, Tsernoglou D (1989) Structure of the membrane-pore-forming fragment of colicin A. Nature 337:93–96

Petosa C, Collier RJ, Klimpel KR, Leppla SH, Liddington RC (1997) Crystal structure of the anthrax toxin protective antigen. Nature 385:833–838

Piredda L, Farrace MG, Lo Bello M, Malorni W, Melino G, Petruzzelli R, Piacentini M (1999) Identification of ''tissue'' transglutaminase binding proteins in neural cells committed to apoptosis. FASEB J 13:355–364

Polekhina G, Board PG, Blackburn AC, Parker MW (2001) The crystal structure of maleylacetoacetate isomerase/glutathione transferase zeta reveals the molecular basis for its remarkable catalytic promiscuity. Biochemistry 40:1567–1576

Qian Z, Okuhara D, Abe MK, Rosner MR (1999) Molecular cloning and characterization of a mitogen-activated protein kinase-associated intracellular chloride channel. J Biol Chem 274:1621–1627

Redhead C, Sullivan SK, Koseki C, Fujiwara K, Edwards JC (1997) Subcellular distribution and targeting of the intracellular chloride channel p64. Mol Biol Cell 8:691–704

Reinemer P, Dirr HW, Ladenstein R, Schaffer J, Gallay O, Huber R (1991) The three-dimensional structure of class pi glutathione S-transferase in complex with glutathione sulfonate at 2.3 Å resolution. EMBO J 10:1997–2005

Reinemer P, Prade L, Hof P, Neuefeind T, Huber R, Zettl R, Palme K, Schell J, Koelln I, Bartunik HD, Bieseler B (1996) Three-dimensional structure of glutathione S-transferase from *Arabidopsis thaliana* at 2.2 Å resolution: structural characterization of herbicide-conjugating plant glutathione S-transferases and a novel active site architecture. J Mol Biol 255:289–309

Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, Drumm ML, Iannuzzi MC, Collins FS, Tsui LC (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 245:1066–1073

Rossjohn J, Feil SC, McKinstry WJ, Tweten RK, Parker MW (1997) Structure of a cholesterol-binding, thiol-activated cytolysin and a model of its membrane form. Cell 89:685–692

Rossjohn J, McKinstry WJ, Oakley AJ, Verger D, Flanagan J, Chelvanayagam G, Tan K-L, Board PG, Parker MW (1998a) Human theta class glutathione transferase: the crystal structure reveals a sulfate-binding pocket within a buried active site. Structure 6:309–322

Rossjohn J, Polekhina G, Feil SC, Allocati N, Masulli M, Di Ilio C, Parker MW (1998b) A mixed disulfide bond in bacterial glutathione transferase: functional and evolutionary implications. Structure 6:721–734

Schendel SL, Xie Z, Montal MO, Matsuyama S, Montal M, Reed JC (1997) Channel formation by antiapoptotic protein Bcl-2. Proc Natl Acad Sci USA 94:5113–5118

Schendel SL, Azimov R, Pawlowski K, Godzik A, Kagan BL, Reed JC (1999) Ion channel activity of the BH3 only Bcl-2 family member, BID. J Biol Chem 274:21932–21936

Schlesinger PH, Blair HC, Teitelbaum SL, Edwards JC (1997a) Characterization of the osteoclast ruffled border chloride channel and its role in bone resorption. J Biol Chem 272:18636–18643

Schlesinger PH, Gross A, Yin XM, Yamamoto K, Saito M, Waksman G, Korsmeyer SJ (1997b) Comparison of the ion channel characteristics of proapoptotic BAX and antiapoptotic BCL-2. Proc Natl Acad Sci USA 94:11357–11362

Shatursky O, Heuck AP, Shepard LA, Rossjohn J, Parker MW, Johnson AE, Tweten RK (1999) The mechanism of membrane insertion for a cholesterol-dependent cytolysin: a novel paradigm for pore-forming toxins. Cell 99:293–299

Sheehan D, Meade G, Foley VM, Dowd CA (2001) Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 360:1–16

Simon DB, Bindra RS, Mansfield TA, Nelson-Williams C, Mendonca E, Stone R, Schurman S, Nayir A, Alpay H, Bakkaloglu A, Rodriguez-Soriano J, Morales JM, Sanjad SA, Taylor CM, Pilz D, Brem A, Trachtman H, Griswold W, Richard GA, John E, Lifton RP (1997) Mutations in the chloride channel gene, *CLCNKB*, cause Bartter's syndrome type III. Nat Genet 17:171–178

Sinning I, Kleywegt GJ, Cowan SW, Reinemer P, Dirr HW, Huber R, Gilliland GL, Armstrong RN, Ji X, Board PG, Olin B, Mannervik B, Jones TA (1993) Structure determination and refinement of human alpha class glutathione transferase A1-1, and a comparison with the mu and pi class enzymes. J Mol Biol 232:192–212

Song L, Hobaugh MR, Shustak C, Cheley S, Bayley H, Gouaux JE (1996) Structure of staphylococcal α-hemolysin, a heptameric transmembrane pore. Science 274:1859–1866

Strange K, Emma F, Jackson PS (1996) Cellular and molecular physiology of volume-sensitive anion channels. Am J Physiol 270:C711–C730

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Tonini R, Ferroni A, Valenzuela SM, Warton K, Campbell TJ, Breit SN, Mazzanti M (2000) Functional characterization of the NCC27 nuclear protein in stable transfected CHO-K1 cells. FASEB J 14:1171–1178

Tulk BM, Schlesinger PH, Kapadia SA, Edwards JC (2000) CLIC-1 functions as a chloride channel when expressed and purified from bacteria. J Biol Chem 275:26986–26993

Valenzuela SM, Martin DK, Por SB, Robbins JM, Warton K, Bootcov MR, Schofield PR, Campbell TJ, Breit SN (1997) Molecular cloning and expression of a chloride ion channel of cell nuclei. J Biol Chem 272:12575–12582

Valenzuela SM, Mazzanti M, Tonini R, Qui MR, Warton K, Musgrove EA, Campbell TJ, Breit SN (2000) The nuclear chloride ion channel NCC27 is involved in regulation of the cell cycle. J Physiol (Lond) 529:541–552

van der Goot FG, González-Mañas JM, Lakey JH, Pattus F (1991) A ''molten-globule'' membrane-insertion intermediate of the pore-forming domain of colicin A. Nature 354:408–410

Wilce MCJ, Board PG, Feil SC, Parker MW (1995) Crystal structure of a theta-class glutathione transferase. EMBO J 14:2133–2143

Zakharyan RA, Sampayo-Reyes A, Healy SM, Tsaprailis G, Board PG, Liebler DC, Aposhian HV (2001) Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione-S-transferase superfamily. Chem Res Toxicol 14:1051–1057