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Review

The CorA family: Structure and function revisited

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Abstract. The CorA family is a group of ion transporters that mediate transport of divalent metal ions across biological membranes. Metal ions are essential elements in most cellular processes and hence the concentrations of ions in cells and organelles must be kept at appropriate levels. Impairment of these systems is implied in a number of pathological conditions. CorA proteins are abundant among the prokaryotic organisms but homologues are present in both human and yeast. The activity of CorA proteins has generally been associated with the transport of magnesium ions but the members of the CorA family can also transport other ions such as cobalt and nickel. The structure of the CorA from *Thermotoga maritima*, which also was the first structure of a divalent cation transporter determined, has opened the possibilities for understanding the mechanisms behind the ion transport and also corrected a number of assumptions that have been made in the past.

Keywords. Membrane proteins, divalent cation transporter, magnesium transport, prokaryotes, topology.

Introduction

Divalent cations are highly important for many biological functions and therefore essential for life. Most of the mammalian cells contain a total cellular Mg^{2+} concentration of 14–20 mM, thus making this ion the most abundant divalent cation within the cells [1]. The biological importance of this cation is highlighted by the critical role in cellular energy metabolism, DNA transcription and replication, protein translation and many signal transduction systems, membrane stability, and hormonal regulation [2–4]. The chemistry of magnesium is also fascinating: it has the largest radius of all cations when hydrated, but is among the smallest when dehydrated. This is a 400-fold increase in volume. It also binds the hydrating waters more strongly than other cations like sodium, potassium and calcium, and this has led to the postulation that activity of magnesium as an enzyme cofactor is mediated through spatial coordination of bound water molecules [5]. These unique features of magnesium lead us to believe that the system for magnesium transport should be rather unique too.

Ion trafficking is a difficult task due to the charged nature of the ion and to the insulating properties of the membrane bilayer, which the ion has to pass. Therefore, this process requires transporters that span the membrane and are specifically designed for this very task. The variety and concentration of the ions needed within the cells of different species demand highly regulated mechanisms for ion selection and gating of the transporters. The cellular concentration of these ions, both free and bound, varies and also depends on

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the cell type. They are, however, vital for signaling. The rate and direction of ion transport is regulated by specialized transporters, so-called divalent metal ion transporters (DMIT). A wide range of DMITs promote such transport either by passive channeling or actively using ATP or other solutes or ions.

Three classes of magnesium transporters have been identified in Bacteria and Archaea, MgtA/B, MgtE and CorA [6–16]. The CorA family is ubiquitous within both Kingdoms indicating that it is their primary magnesium transporter. The CorA family is the only one of the three families that has been studied extensively. It is known to be the major system for magnesium transport in bacteria. The understanding of the structure and function of this family reached a higher level when the 3-D structure of a CorA homologue was reported recently by three groups independently [17–19]. In this review we aim to discuss the current understanding about the proteins in the CorA family with especial emphasis on the structure and function. We highlight the existence of two, so far neglected, groups within the CorA family that have structural differences, which most probably result in functional differences. We also briefly review the current knowledge about the eukaryotic homologues.

Prokaryotic CorA

The first indications of a system for transport of magnesium across the biological membranes were brought to attention in 1969 by Simon Silver, who published the discovery of the system in *Escherichia coli* at the same time as Lusk and Kennedy [20, 21]. Later in 1971, Nelson and Kennedy suggested that the same system for magnesium uptake in *E. coli*, discovered by Silver, Lusk and Kennedy, would also transport cobalt, nickel and cadmium, and that cobalt uptake would also inhibit magnesium uptake by the cell [22]. Fifteen years later, the gene for this system, referred to as *corA*, was cloned from *Salmonella typhimurium* for the first time [10].

Classification

The CorA family genes are found in prokaryotic and eukaryotic cells [23]. The sequence homology between the family members is lowest near the N terminal, but gradually increases towards the C terminus, where the transmembrane (TM) domain is located. At this end the most characteristic signatures of the CorA proteins, MPEL and YGMNF, are found, both positioned in the loop between the transmembrane helices.

The latter is the most conserved region through the whole family and is also found in the eukaryotic homologues, shortened to GMN [24]. This sequence is believed to be essential for function and forms the signature motif of the entire family. Mutations in the GMN motif are known to abolish Mg^{2+} transport, but the naturally occurring variants GVN and GIN may be associated with the transport of other divalent cations. For instance, a distant CorA homologue in *Salmonella typhimurium*, named ZntB, has experimentally been characterized as a putative zinc transporter, possibly mediating export of Zn^{2+} and Cd^{2+}, but unable to transport Mg^{2+} [25]. ZntB shows the basic CorA type of structure with two C-terminal TM stretches, but the GMN motif is altered to GIN.

Another class of CorA proteins has also been reported, termed 'CorA-II', which lack the characteristic MPEL motif and is suggested to transport divalent cations out of the cell [16]. Based on this classification, *Methanococcus janaschii* would fall into this category. Kehres and Maguire [16] believe this class may have diverged from CorA relatively early in the evolution. This class of CorA is not discussed in this review.

We performed sequence alignment of CorA proteins from 31 different organisms (Fig. 1). A closer look at this alignment reveals two groups within the CorA proteins, A and B, where a number of amino acids are well conserved within one group whereas the other group has the same degree of conservation of other amino acids. As discussed in the next section, some of the differences are among amino acids involved in metal binding and therefore highly important for the function. Both from the sequence alignment and the phylogenetic tree (Fig. 2), it is obvious that CorA from *T. maritima*, for which a 3-D structure is known [17] belongs to group A, whereas CorA from *E. coli* and *S. typhimurium*, which are the most well-studied CorA proteins, belongs to group B. The former has also more similarities to the *M. jannaschii* and may be more related with the CorA-II. The idea of subgroups within the CorA family is supported by disagreements between the CorA structure from *T. maritima* and the extensive work performed by Maguire and co-workers [26–29], as well as the recent work reported by Wang *et al.* [30], on the *E. coli* and *S. typhimurium* homologues. This is further discussed in the next sections.

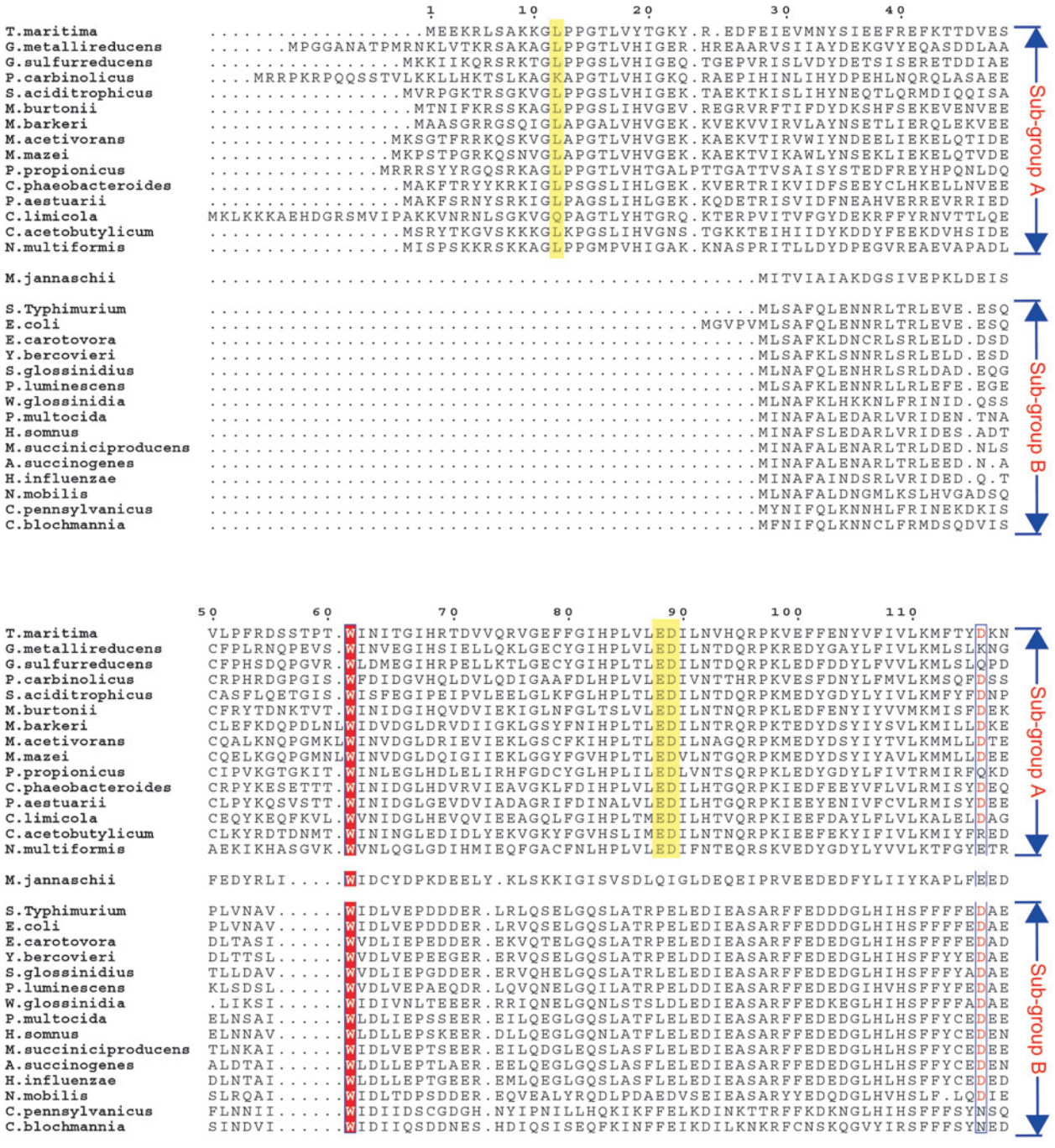

Figure 1. Sequence alignment of CorA from 31 different organisms. Sequences in group A have been highlighted to address their role identified in the crystal structure from, *T. maritima:* M1 and M2 coordination are highlighted as yellow, putative Cl^- coordination is in blue and the aspartates making the 'aspartate ring' are in green. The position of TM1 and TM2 seen in the crystal structure are shown with yellow bars, and the predicted TM1-TM3 from, *E. coli* and, *S. typhimurium* are shown with blue bars.

Structure and topology

The only available structure of any divalent cation transporter, *i.e.*, the CorA from *T. maritima*, was provided almost simultaneously by three independent research groups, one presenting a high-resolution structure [17] and the others reporting similar structures at lower resolution [18, 19]. For a detailed description of the structure of this protein, we refer to the original reports as well as a recent review [31]. Here, we describe the most important features of the structure and discuss how general these features are within the CorA family.

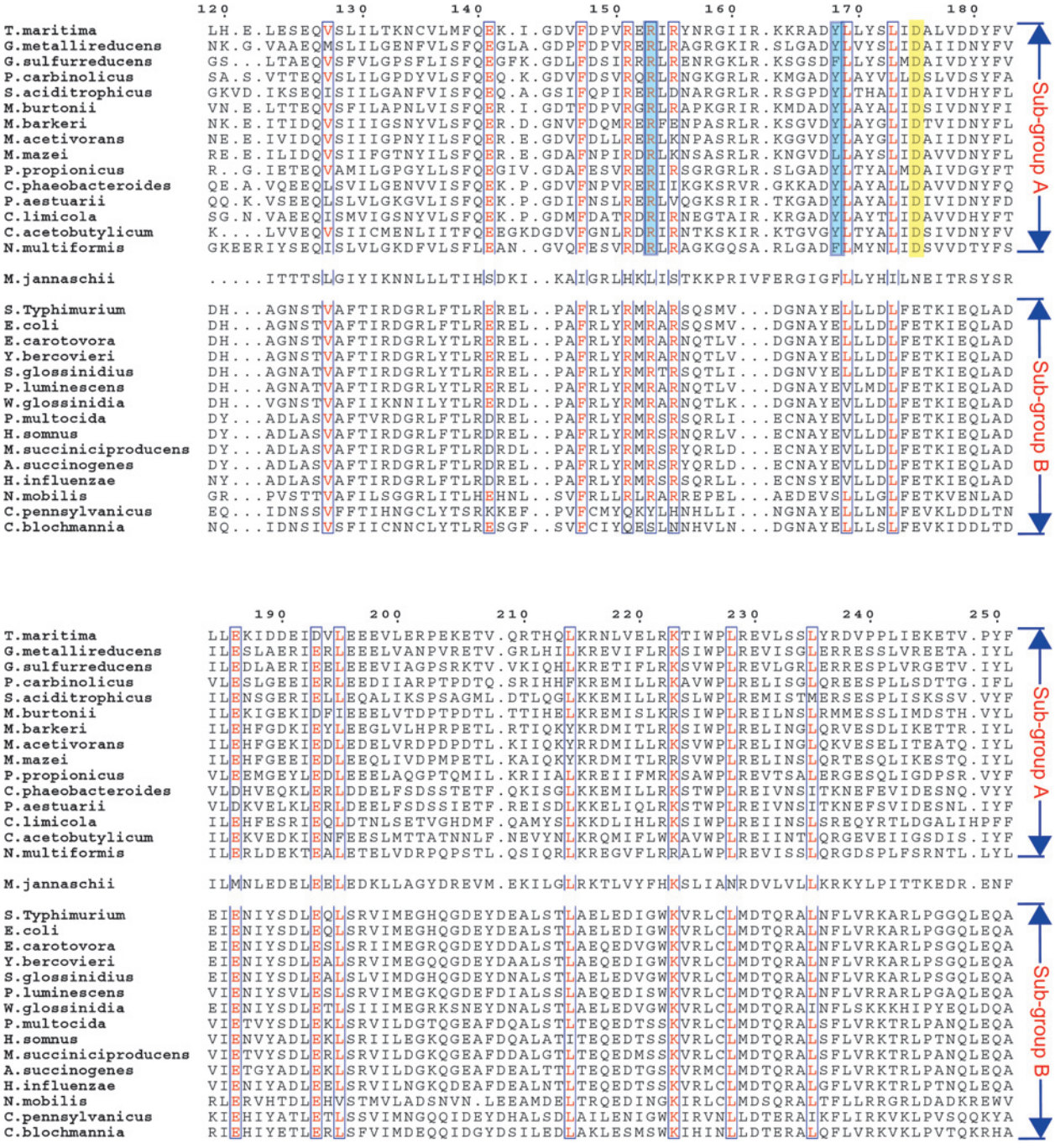

Figure 1. *(continued)*

The structure reveals a funnel-shaped homopentamer with two transmembrane helices, TM1 and TM2, where TM1 creates the inner cavity and is surrounded by a ring made by TM2 from each monomer (Fig. 3). Two metal binding sites, M1 and M2, were identified in the structure (Fig. 3b and c) to which Mg^{2+}, Co^{2+} and Ca^{2+} can bind, although the latter is not yet known as a substrate for CorA and further biochemical as well as biological data are required to support the relevance. M1 was identified in all three structures and was shown to be coordinated by the carboxylates from D89 and D253. M2, on the other hand, was only seen in the structures reported by Eshaghi *et al.* [17] and Payandeh and Pai [19]. The latter has identified four amino acids coordinating the bound Ca^{2+} at the M2 site: E88, D175, D179 and H257. However, Ca^{2+} is not known as a CorA substrate or inhibitor and is more tolerant when it comes to coordination numbers and

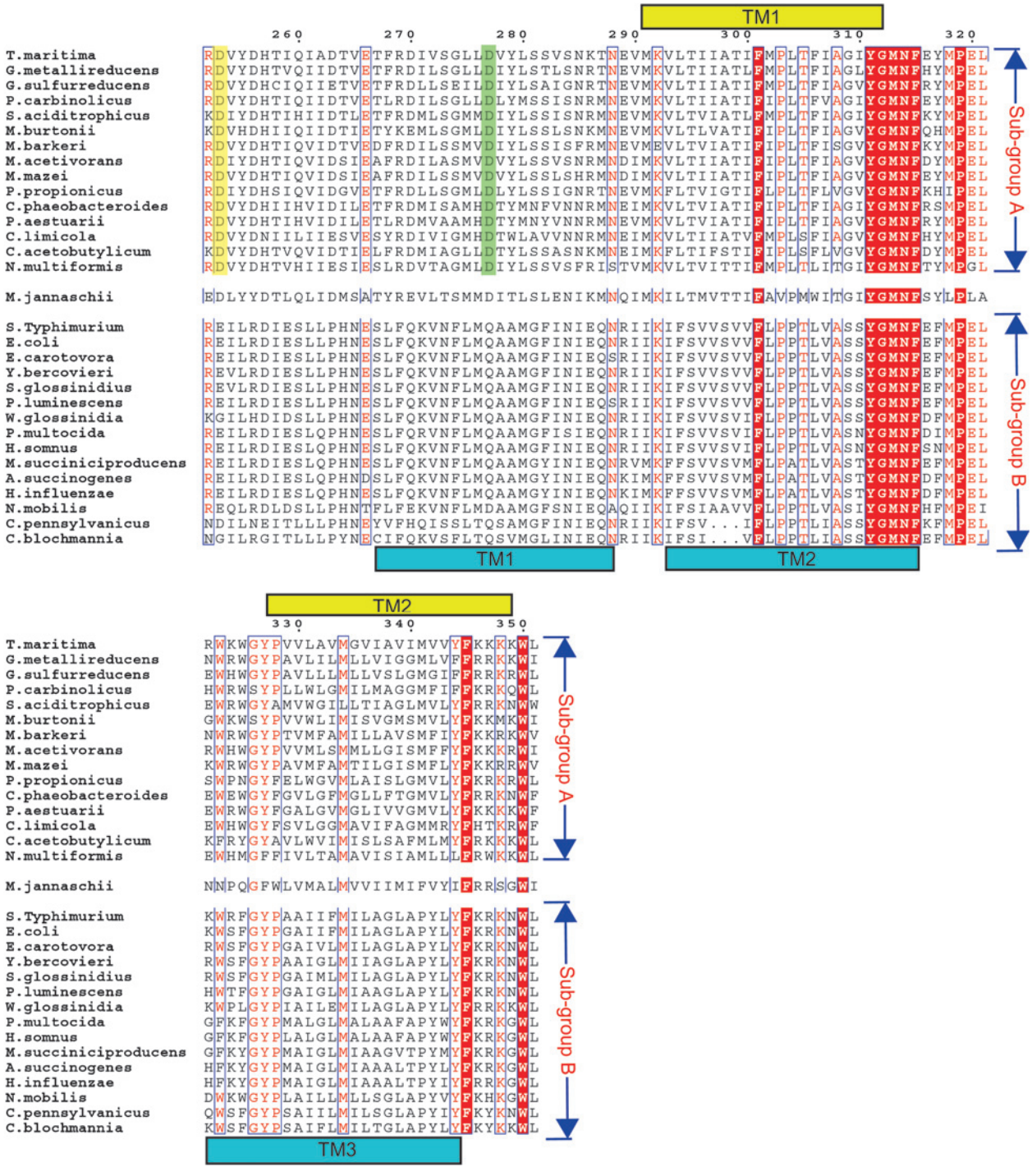

Figure 1. *(continued)*

can adopt a 6, 7, 8 and a less frequent 9 coordination. The octahedral coordination sphere is also highly flexible [32]. This is compared to the much more constrained coordination sphere of Mg^{2+}, which is hexacoordinated and is also the natural substrate of CorA. The most detailed structure of CorA, reported by Eshaghi *et al.* at 2.9 Å resolution, presents both Mg^{2+} and Co^{2+} bound in the M2 site. Although the exact coordination sphere cannot be identified, these ions seem to be coordinated *via* a network of water molecules by the carboxylates from E88, D175 and D253 and also, interestingly the carbonyl from L12. L12 is part of the N-terminal domain that exists only in the group of *T. maritima* CorA (Fig. 1) and is highly

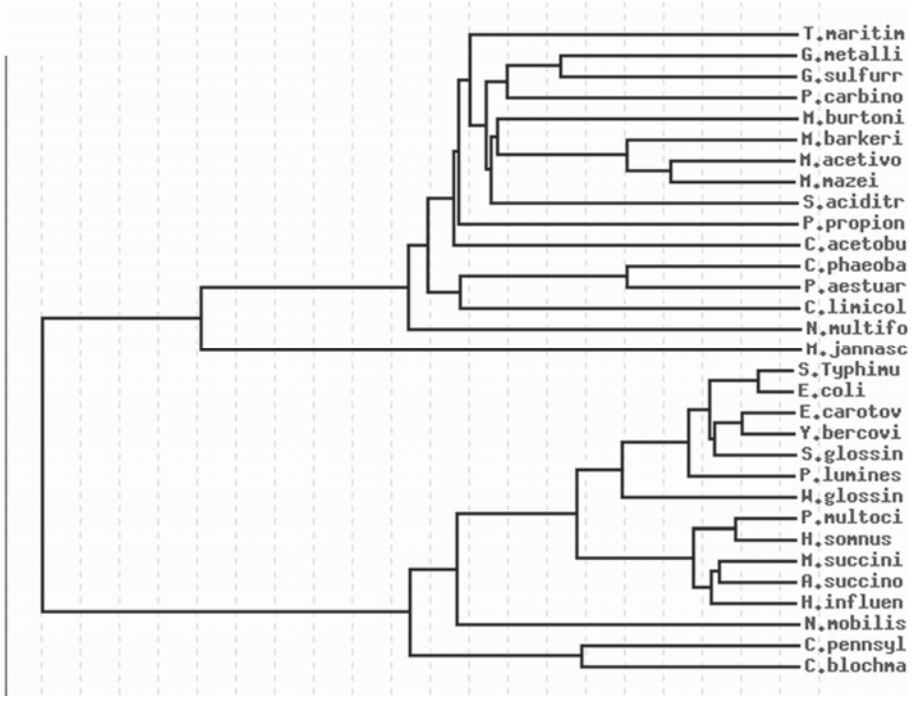

Figure 2. Phylogenetic tree showing the presence of two groups within the CorA family.

conserved. Sequence alignments of this domain only highlight the conservation of the GLPPG sequence. In the high-resolution structure of CorA, the prolines in this sequence seem to be important for creating a bend, which is important for stabilizing the conformation of this region.

Since the metals are coordinated both by carboxylic side chains and carbonyls from the main chains of adjacent monomers, one can postulate that this coordination may add to the stability of the oligomer. However, we have some preliminary results showing no destabilization of the oligomer following metal depletion (unpublished data). Nevertheless, the *T. maritima* CorA is highly protected against proteolysis in the presence of Mg^{2+}, Co^{2+}, Ca^{2+} and Ni^{2+} [19], supporting that bound divalent cations increases the stability of the entire protein possibly through the stabilization of the N terminus.

The large hydrophilic portion of the N-terminal domain forms the funnel opening. The opening becomes smaller towards the C terminus and reaches the minimum at the position of the aspartic acid 277 (D277). At this position, the carboxylic side chains from each monomer are pointed towards the pore and together create a characteristic negatively charged ring, the 'aspartate ring' (Fig. 3b). Interestingly, bound Mg^{2+} as well as Ca^{2+} have been identified within coordinating distance of this ring. Thus, this aspartate ring is likely to have a regulatory function or to be involved in substrate selection or substrate dehydration. A similar arrangement has previously been observed in TolC [33]. TolC is a trimeric membrane protein that mediates multidrug efflux and protein export from bacteria [34]. An aspartate ring, composed of three aspartic acid residues has been observed in the crystal structure of this protein [33, 35]. Monovalent cations can pass through the ring, but divalent cations inhibit the ion passage [35, 36].

The loop connecting TM1 and TM2 contains the conserved CorA signature motifs YGMNF and MPEL. This loop seems to be rather flexible and its structure cannot be determined from any of the available crystal structures. An indication of the possible dehydration/rehydration site can, however, be seen where the carbonyls from backbone of Y311 and G312 (from YGMNF) as well as G309 are pointing towards the center of the pentamer. This could create a well-suited charged environment at the entry point of the translocation channel [17] (Fig. 3d). There is currently no evidence about the orientation of the protein in the membrane. However, it seems more likely that both N and C termini point to the cytosolic side. This suggestion is supported by the 'positive-inside rule' [37] and the 'hairpin' topology [38].

Structural discrepancies between the two groups

The crystal structure of *T. maritima* CorA represents the only data available from this group of CorA. On the other hand, there is a huge amount of data and

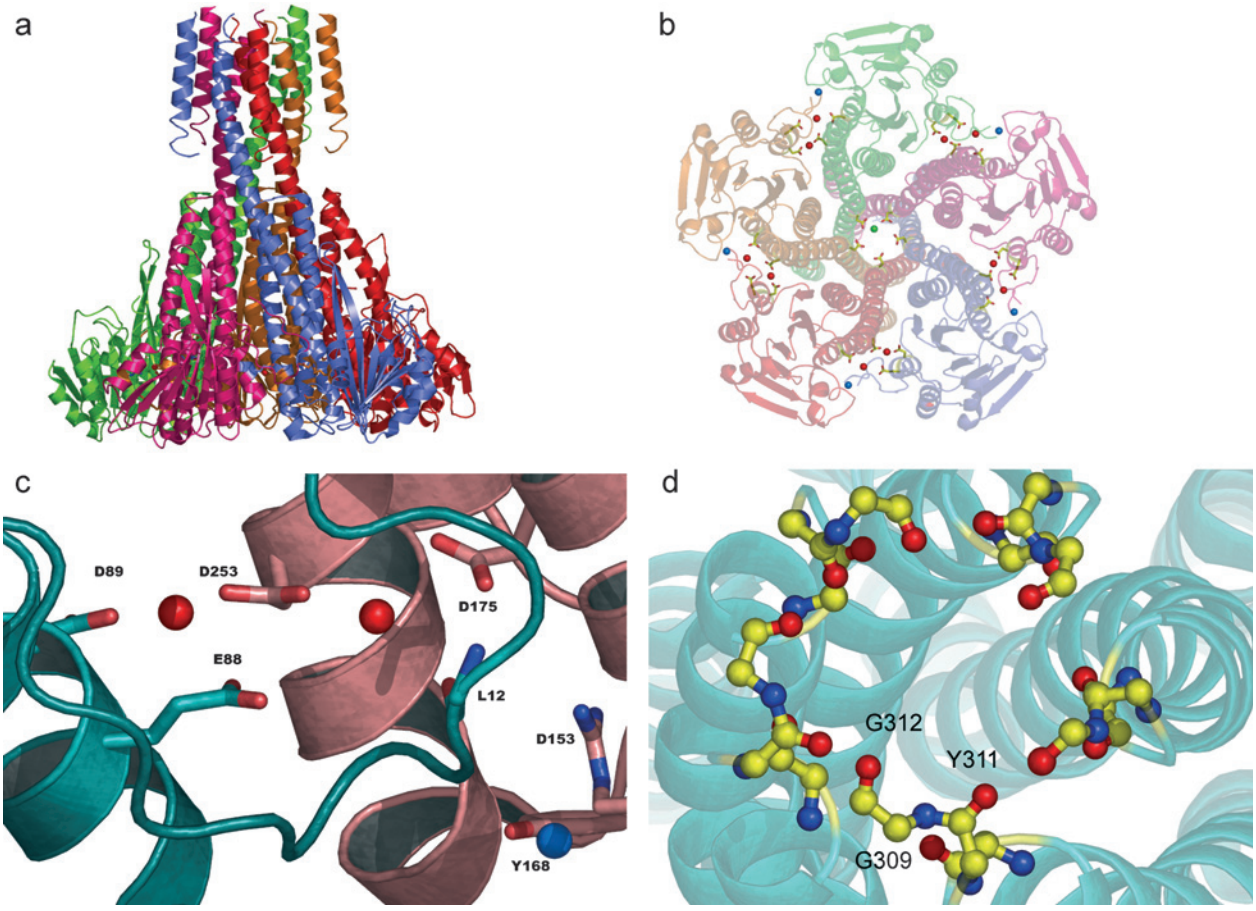

Figure 3. Crystal structure of CorA from, *T. maritima.* (*a*) Overall structure from the side showing the transmembrane helixes TM1 and TM2 on top and the large hydrophilic domain at the bottom; (*b*) overall pentameric arrangement as seen from the cytoplasmic side. The Mg^{2+} ions in M1 and M2 are colored red. Putative Mg^{2+} ion near the D277 aspartate ring in green and Cl^- ion depicted in blue; (*c*) close-up of the M1 and M2 metal sites showing Mg^{2+} in red and the putative Cl^- ion in blue; (*d*) the carbonyls from the main chain at the periplasmic entrance possibly creating a negatively charged environment for an incoming ion.

results derived from molecular biology and biochemical experiments performed on CorAs from *E. coli* and *S. typhimurium*, which belong to group B. As there is no high-resolution structure available from this group, the obvious structural differences between the two groups concern the topology. Maguire and co-workers reported in 1993 [26] that the *E. coli* CorA contained three TM helices with the large hydrophilic N-terminal domain facing the extracellular domain. Later, they even identified the functionally important amino acids in TM helices 2 and 3 using *S. typhimurium* CorA [27, 28]. Even though the hydrophobic analysis of the amino acid sequences of CorA proteins, from any group, predicts the existence of two TM helices positioned at the C-terminal end with both the N and C termini facing the intracellular domain (using public servers TMHMM, TMPred or SMART), the topology suggested by Maguire and colleagues was generally accepted and CorA proteins were referred to contain three TM helices at the C-terminal end and to have the hydrophilic N-terminal exposed to the periplasmic side.

The Maguire group also suggested CorA proteins to be tetramers rather than pentamers based on cross-linking experiments on CorA from *E. coli.* However, after the crystal structure of CorA was published, the assumption that the structure would be representative for the whole family seemed obvious and Maguire abandoned his former results in two recent reviews [31, 32]. However, a closer analysis of the amino acids involved in metal binding identified in the crystal structure and comparing with CorA amino acid sequences from the other group, clarifies the differences between the two groups even more. The N-terminal extension of group A containing L12 involved in M2 coordination is missing in group B. This difference is probably the major reason for the lack of homology in this region in the entire CorA family. When on its own, the region is well conserved within group A. These amino acids are interestingly found around L12 (Fig. 1).

Another important difference between the two groups concerns the very well-defined 'aspartic ring' made by D277: the negatively charged aspartic acid in group A is replaced with the uncharged, and highly conserved, glutamine in group B. It is highly questionable whether this polar and amide-containing residue can functionally replace the carboxyls of the aspartate ring. In fact, the glutamine in group B falls in the middle of the first TM helix predicted in *E. coli* and *S. typhimurium* (Fig. 1). The sequence conservation in the region of the predicted TM1 is very high within group B, but less profound in the same region within group A. Also the total polarity of this region is higher in group A than group B. Recently, Wang *et al.* [30] reported electron microscopy data as well as biochemical and biophysical data from *E. coli* CorA showing, in contrast to the crystal structure of *T. maritima* CorA, the existence of three TM helices and a tetrameric CorA, thus confirming the previous results obtained by Maguire and coworkers. On these premises, two possible topologies could be suggested for group B CorA (Fig. 4). The first alternative suggests that what was previously known as TM1 actually is not completely transmembrane but rather peripheral to the membrane. Thus, both terminals would face the same side. The second alternative is the previously suggested three TM helix scenario, leaving N and C termini on opposite sides. What is interesting with this alternative is that the conserved loop would appear at the same side of the large hydrophilic N-terminal domain. Apparently, only a structure analysis of a group B CorA would clarify this.

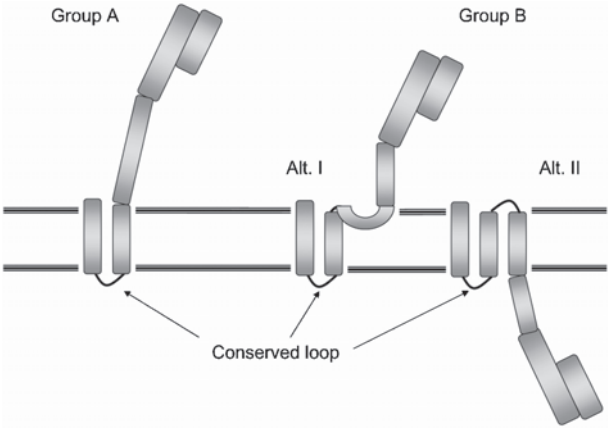

Figure 4. Possible topologies of group B CorA and the comparison to group A.

Physiology

Since the discovery of CorA, most of the biochemical characterization of this family has been performed on homologues from *E. coli* and *S. typhimurium*. Transport parameters have been well established for CorA systems from *E. coli*, *S. typhimurium* and *Haemophilus influenzae*, all belonging to group B. They all mediate the influx of Mg^{2+}, Co^{2+} and Ni^{2+}, with the affinities of 15–20 μM, 20–40 μM and 200–400 μM, respectively [10, 30, 39]. In *S. typhimurium*, the influx of Mg^{2+} is linear for a short time of 15–20 s and then stabilizes. However, the uptake of both Co^{2+} and Ni^{2+} is linear for at least 15 min. One explanation would be that CorA is the only system for controlling the intracellular levels of Mg^{2+} by coupling influx and efflux. This explanation is strengthened by the fact that efflux of only Mg^{2+} (and not Co^{2+} and Ni^{2+}) has been reported for these proteins, and no Mg^{2+} efflux can be observed in the absence of a functional CorA [40].

Biochemical and functional characterization of group A CorA is completely lacking in the literature. The identification of bound metal ions Mg^{2+}, Co^{2+} and Ca^{2+} represents the only available data on the substrates of this group. One can speculate about the role of Ca^{2+} since it is not considered to be a CorA substrate. Its promiscuous binding also differs from that of Mg^{2+}. However, the presence of this divalent cation at M1 and M2 as well as near the D277 aspartate ring implies the importance of these sites. Whether Ca^{2+} is a natural substrate/regulator of group A requires biological evidence. The Co^{2+} binding was achieved by soaking experiments during crystallization procedures and showed that Co^{2+} could replace Mg^{2+} at M1 and M2 at a fifth of the concentration [17]. These are the only data available from group A showing substrate competition, which suggest that group A CorAs have a higher affinity for Co^{2+} than Mg^{2+}. Nevertheless, further biochemical and/or biophysical characterizations are needed for identifying other substrates and their affinity to this group of CorAs.

Ion transport

Is the metal ion substrate transported in a fully hydrated, partially hydrated or totally dehydrated form? How is the ion selected? Does CorA act as a channel or does it use a counter ion to promote the transport? These questions are still unanswered and the crystal structure of CorA from *T. maritima*, although identifying metal binding sites that are potential regulatory sites, does not point to a clear mechanism. This is because there is no metal ion present in the channel. This, in turn, makes it difficult to speculate about how a substrate would be coordinated by the amino acids in the channel. Metal ions are usually coordinated by oxygen from either carboxylic

side chains or the main chain carbonyls. For example, based on the structures of the potassium channel KcsA and the Na^+/K^+ channel, it is obvious how the metal ion substrates are coordinated by the main chain carbonyls [41–44]. The entire passage through the CorA membrane is devoid of negatively charged amino acids, and the main chain carbonyls are not in such a position that they could coordinate the substrate. Also, the structure reveals an apparent closed state. Thus, one can only speculate on how the ion is transported through the channel. A model for the open state has been presented by Payandeh and Pai [19] and is based on the superposition of the crystal structure of the soluble CorA domain from *Archaeoglobus fulgidus* (a close homologue of *T. maritima* CorA and thus a member of group A) on the crystal structure of full-length CorA from *T. maritima.* Based on this model, a ~180° rotation of helix α6 around α7 would cause an opening of the pore allowing ion translocation. This rotation would be a consequence of substrate dissociation from M1 and M2, as a result of low metal ion concentration within the cell, allowing influx. However, structural data are required to prove this a plausible model. One should also remember that the CorA group B can both import and export Mg^{2+} from the cell. Therefore, high substrate concentration within the cell should presumably also cause pore opening. The Mg^{2+} efflux would require high ion concentration outside the cell, which is quite rare and is found in certain milieus such as sea water. As for the group A organisms, such as *T. maritima* that exists in extreme milieus, unusually high extracellular metal ion concentrations may occur. As a matter of fact, many of the organisms in this group exist in extreme environments. Therefore, CorAs in this group may rather perform efflux than influx. The presence of the 'aspartate ring' at the intracellular entrance of the pore may be such an indication, as it is missing in the group B CorA and the differences between the two groups are vast in this region. The 'aspartate ring' and the carbonyls from the conserved loop at the periplasmic side indicate that ion can readily be transported in both directions.

The mode of transport is a quite unclear issue. A putative chloride ion positioned near M2 site, coordinated by Y168 and R53 has been assigned in the high-resolution structure (Fig. 3c) [17]. Due to the close location of this negatively charged ion to the regulatory metal binding site, one could speculate that it could act as a counterion during the metal ion transport, suggesting the group A CorAs to be a symporters.

Eukaryotic homologues

In recent years, functional homologues have been characterized in the inner mitochondrial membrane of yeast and mammals (the MRS2/LPE10 type), in the plasma membrane of yeast (the ALR/MNR type) and extend to the model plant *Arabidopsis thaliana* [23, 45–47]. All these proteins are characterized by the conserved GMN motif at the end of the first of two conserved TM domains near the C terminus. MRS2 is nuclear encoded and after translation it is transported to the inner mitochondrial membrane. The Mg^{2+} transport through this channel is believed to be electrophoretic, *i.e.*, driven by the inside negative membrane potential [48]. Its dysfunction reduces the levels of Mg^{2+} in mitochondrial matrix and as a consequence group II splicing is reduced [49–51]. ALR increases tolerance to Al^{3+} and Ga^{3+} ions when overexpressed in *S. cerevisiae* while increasing strain sensitivity to Zn^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Ca^{2+}, and La^{3+} ions [52]. ALR proteins are also suggested to have a central role in cell survival in a cadmium-rich environment [53].

Programs for predicting TM stretches consistently predict two TM helices near the C termini of all of these proteins. This topology is supported by biochemical analyses of MRS2 and LPE10 [45, 51] and ALR [54], suggesting that both ends of the proteins reside in the mitochondrial matrix. Cross-linking studies imply that ALR is an oligomer [54] and MRS2 functions as a pentamer in the membrane [48]. All these data strongly suggest that the eukaryotic CorA homologues have the same topology and overall structure as *T. maritima* CorA.

Despite generally low sequence similarity, individual proteins can functionally complement each other over large phylogenetic distances. For instance, both human MRS2 and *S. typhimurium* CorA can restore Mg^{2+} uptake activity in *Saccharomyces cerevisiae* with inactive MRS2 [45, 51, 55].

Concluding remarks

The CorA family is universally distributed within prokaryotes and is responsible for transport of divalent cations, in particular Mg^{2+}. Functional homologues exist in eukaryotes, all containing two C-terminal TM helices and the GMN signature motif from CorA.

Recent advances in understanding the structure and function of the CorA family have been dramatic, and detailed structural information is now added to previously obtained functional and biochemical data. However, there seem to be a number of discrepancies

between the available information. A closer analysis of the available data shows that these discrepancies are a result of deviations in the CorA family. The CorA family can be divided into two groups with certain differences. This has become apparent after the availability of the crystal structure from *T. maritima* homologue that we have classified as a member of group A. On the other hand, all biochemical and functional data have originated mainly from *E. coli* and *S. typhimurium,* belonging to group B. It is tempting to extrapolate the biochemical results obtained from group B to group A. However, until a high-resolution structure from sub-group B is reported, such assumptions are only speculative, due to the number of structural differences clarified in this review.

The crystal structure reveals a pentameric membrane protein channel featuring a possible ion discriminating aspartate ring at the cytoplasmic entrance of the pore and two distinct cytoplasmic metal binding sites per monomer, which could have regulatory roles. The aspartate ring does not seem to be present in the other group. Instead, the corresponding region may be buried (fully or partially) in the membrane. To provide a more comprehensive understanding of the bacterial members of this transporter family, structural information on the *S. typhimurium* and/or *E. coli* CorA proteins, as well as detailed biochemical characterization of the *T. maritima* CorA will be essential.

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