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Organization of P-Type ATPases: Significance of Structural Diversity†

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The P-type ATPases form a large family of more than 50 membrane proteins, which are responsible for the active transport of a variety of cations across cell membranes. Translocation of cations against their electrochemical potential gradient is achieved by utilizing the energy of hydrolysis of the terminal phosphate bond of ATP. Phosphorylation of the aspartic acid in the invariant sequence DKTG during turnover distinguishes P-type ATPases from V-type and F_oF_1-ATPases. This article is focused on the structural diversity among P-type ATPases, and it relates the general organization of the P-type ATPase molecule to the type of transported cation. Structural variations among the ATPases as well as striking differences in the properties of transported cations suggest that details of the molecular mechanisms underlying the translocation of different cations are not identical. Presently little experimental data are available on properties of the majority of the newly discovered ATPases, and it is evident that assumptions regarding commonality in their structures and mechanisms require thorough testing. In particular, we hope to show that current views on structural organization of P-type ATPases require reassessment in order to reconcile the idea of common overall mechanism with their structural diversity.

A. GENERAL FEATURES OF P-TYPE ATPASES

It is not an easy task to describe the structural organization of a "typical" P-type ATPase as there are considerable differences among the members of this family. Some of the P-type ATPases function as two- or multisubunit complexes, others seem to work well employing just a single subunit. When one considers the catalytic subunits, of which the conserved regions have been well described (Serrano, 1988), the molecular weights differ dramatically from 72 kDa for Cd-transporting bacterial ATPase (Nucifora et al., 1989) to 200 kDa for ATP1−ATPase of Plasmodium (Krishna et al., 1993). The reviews by Taylor and Green (1989) and Serrano (1988) describe regions of homology in P-type ATPases and speculate on their functional significance. Here we briefly summarize that information, leaving the reader to obtain specific details in the cited literature.

Several regions of high homology have been revealed in the structure of the large catalytic subunit; these include the phosphorylation site (DKTGS/T), the TGES/A motif located in a conformationally flexible loop, the TGDN motif in the putative ATP-binding domain, and the MXGDGXNDXP sequence that connects the ATP-binding domain to transmembrane segments that are involved in ion binding and translocation. Chemical modification with residue-specific reagents or substrate analogs based upon ATP [for review see Pedemonte and Kaplan (1990)] led to the conclusion that most of the ATP-binding site is shaped by amino acid residues in the largest cytoplasmic loop. It is becoming increasingly clear that the moiety involved in coordination of transported cations is formed by transmembrane segments (Clarke et al., 1989; Capasso et al., 1992). The exact number of transmembrane segments in each of the P-type ATPases is still a matter of debate and experimentation, and it certainly varies among the different members of the family.

B. TRANSMEMBRANE TOPOLOGY AND DOMAIN STRUCTURE OF P-TYPE ATPASES CORRELATE WITH THE TYPE OF TRANSPORTED CATIONS

It is important to stress that the often quoted distinction between bacterial and eukaryotic ATPases as two separate

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groups among this family is probably incorrect and misleading. We will show that a classification based on the type of transported cations (heavy-metal or non-heavy-metal) provides a more consistent picture for the P-type ATPase family. This classification is independent of the organism from which the enzymes are isolated or identified and allows us to delineate common and specific domains much more readily. This point is illustrated on Figure 1, which shows that hydropathy profiles of the catalytic subunits of P-type ATPases fall into two distinct groups. ATPases involved in transport of heavy metals (P_1-ATPases) have very similar hydropathy profiles which differ dramatically from those of ATPases which transport non-heavy-metals (P_2-ATPases). KdpB-ATPase from *Escherichia coli* (P_3-ATPase) shares structural features with both types.

P_1: Heavy-metal-transporting P-type ATPases include bacterial ATPases Cd-ATPase (Nucifora et al., 1989), CopA and CopB (Odermatt et al., 1993), fixI (Kahn et al., 1989), *Synechococcus* ATPase (Kanamaru, 1993) and others, human Cu-transporting ATPases [Menkes and Wilson disease-associated proteins (Vulpe et al., 1993; Tanzi et al., 1993; Bull et al., 1993)] and a putative Cu-transporting ATPase of yeast (Fu et al., 1994). All these proteins have 6−8 putative transmembrane segments with only one pair of membrane segments located C-terminal to the cytoplasmic ATP-binding domain (Figure 1). They all have a very hydrophobic region, prior to the sequence TGES/A, that may correspond to two pairs of transmembrane crossings, while P_2- and P_3-ATPases have only one pair of transmembrane segments in this region (Figure 1). Whether or not P_1-ATPases have an additional transmembrane hairpin prior to the TGES/A motif and what its role might be in heavy-metal transport is an intriguing question that awaits further study. The most striking feature of these ATPases is the presence of 1−6 "metal-binding" motifs, GTMCXXC or M/HXXM DHS/GXM at the N-terminus of the molecule (Silver et al., 1993; Petrukhin et al., 1994; Bull & Cox, 1994). Differences in the number of times this metal-binding motif is repeated provide the basis for the enormous variation in molecular weight among these proteins.

P_2: Non-heavy-metal-transporting P-type ATPases have a catalytic subunit of molecular weight around 100 kDa with 8−10 putative transmembrane segments (Figure 1). In contrast to heavy-metal-transporting ATPases, only one pair of highly hydrophobic segments is located before the TGES/A motif while four additional transmembrane segments are seen in the C-terminal potion of the molecule (compare plots on Figure 1). This large group of P-type ATPases can be further divided into two subgroups: (a) heterosubunit Na,K-ATPase and H,K-ATPase, which exchange intracellular Na^+ or H^+ respectively for extracellular K^+ and which are composed of the catalytic α-subunit and a glycosylated β-subunit; (b) monosubunit ATPases [Ca-ATPases of SR and plasma membrane, Mg-ATPases of *Salmonella* (MgtA and MgtB), H-ATPase of fungi and plants, and others], which transport divalent cations or protons from the cytoplasm into the intracellular compartments or into the extracellular milieu. (Role of Mg-ATPases in the efflux of any cations has not been shown.)

While this broad classification of P-type ATPases provides a very good correlation between major structural features and the type of transported cation (heavy-metal versus non-heavy-metal), knowlege of primary structure alone is often not sufficient for more refined groupings of P-type ATPases within these large subfamilies. The well-known dissimilarity sequences of the Ca-ATPases of SR and the plasma membrane along with site-directed mutagenesis data [for example, Clarke et al. (1989) and Andersen and Vilsen (1995)] illustrate clearly that the cation-specific coordination center is formed from separated segments of the protein, rather than by a continuous recognizable linear sequence. Homology of primary structure and similarities in domain composition apparently reflect the general aspects of the overall mechanism of P-type ATPases, for example the way in which cation recognition and binding are linked to the activation of phosphorylation from ATP.

P_3: KdpABC-ATPase is similar to the other non-heavy-metal ATPases in that its catalytic subunit KdpB has a single pair of transmembrane fragments before the TGES sequence and no metal-binding motif at the N-terminus. KdpB is also essentially shorter in its C-terminal region, resembling in this part of the molecule the heavy-metal-transporting ATPases (Figure 1). However, in contrast to bacterial heavy-metal-transporting P-type ATPases, which have been shown to be able to carry out cation extrusion as monosubunit proteins (Tsai & Linnet, 1993; Nucifora et al., 1989), KdpB-ATPase is a part of a complex molecular machine which supports active potassium influx and does not function as a single unit (Altendorf et al., 1992).The exact numbers of transmembrane crossings in KdpB-ATPase remains to be determined. Careful inspection of the hydropathy profile in the C-terminal part of the molecule and particularly sequence comparison with analogous regions of other P-type ATPases provide enough room for two, three, or even four transmembrane segments in this region.

C. CORE STRUCTURE FOR P-TYPE ATPASE

This broad classification allows us to dissect the structure of all P-type ATPase molecules into a core or minimal unit and additional domains, which are specific for the various groups (Figure 2). The core protein comprises three pairs of transmembrane segments: one before the TGES/A sequence, one just before the phosphorylation site (DKTG motif), and one immediately after the ATP-binding domain (MXGDGXNDXP motif), with a series of connecting loops. It is likely that all the components directly involved in the translocation of cations coupled to ATP hydrolysis are located within this core structure. In fact, the simplest Cd- and Cu-ATPases have only a heavy-metal-binding motif and an extra pair of hydrophobic segments in the N-terminal part of the molecule in addition to this core structure.

If indeed this structure satisfies the requirements for the ATP-coupled active transport of cations, then the obvious question arises as to the role of the other nonconserved domains in the structures of the members of this family. In some of the P-type ATPases up to 30%−40% of the entire sequence is composed of such additional structure.

D. ROLE OF NONCONSERVED REGIONS AND VARIABLE DOMAINS

1. N-Terminal Domain Involvement in Cation-Dependent Regulation of ATPase Activity. The N-terminal segments are remarkably different for the three major groups of P-type ATPases. The cytoplasmic sequence at the N-terminus of KdpB is rather short, while all other non-heavy-metal-

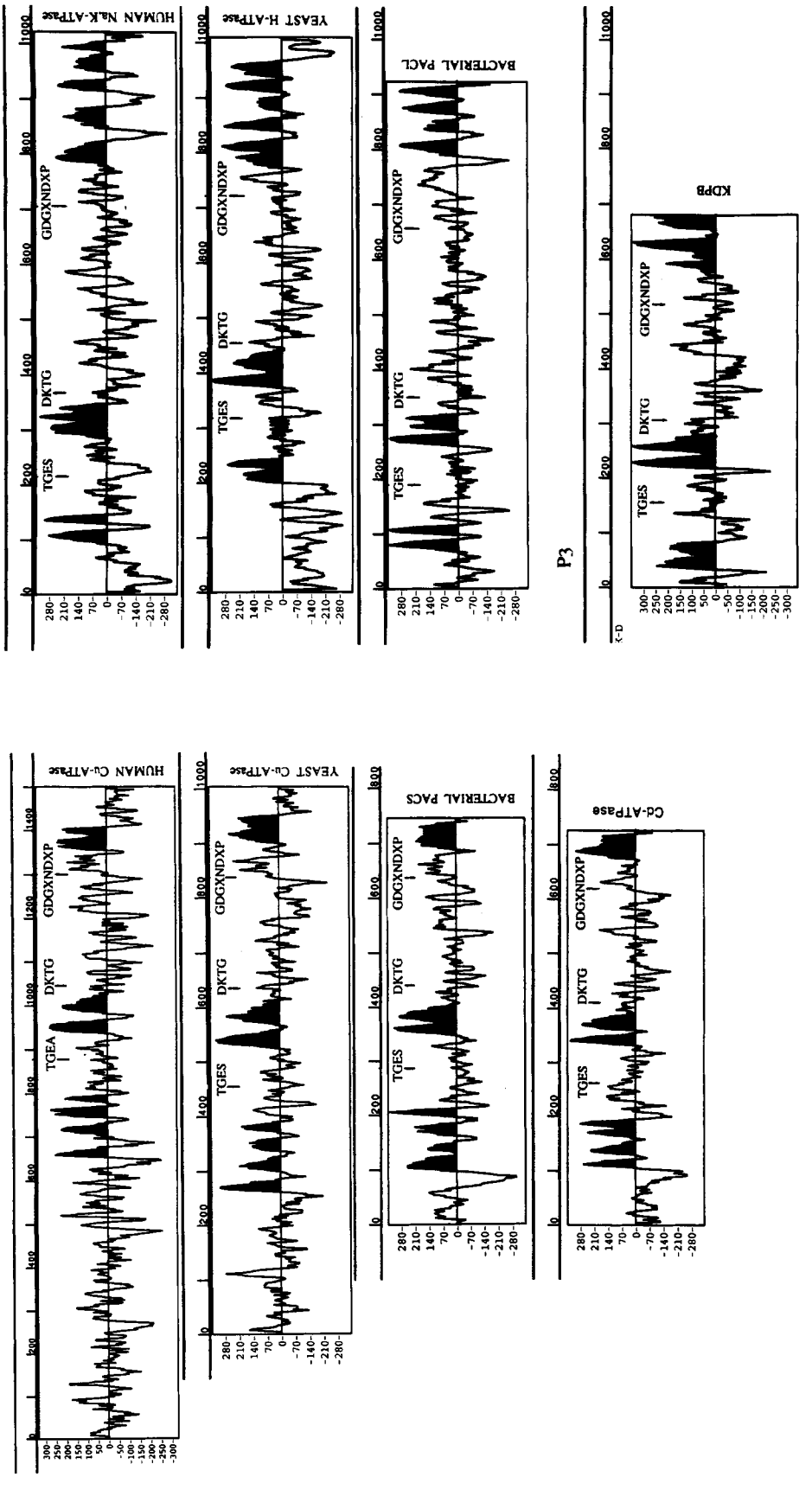

FIGURE 1: Comparison of the hydropathy profiles for the different groups of P-type ATPases: heavy-metal-transporting ATPases (P_1), non-heavy-metal-transporting ATPases (P_2), and KdpB (P_3). GeneBank accession numbers: human Na,K-ATPase (P050123), human Cu-ATPase (L06133), yeast H-ATPase (M60471), yeast putative Cu-ATPase (L 36317), *Synechoccocus* ATPases (PACL-D16436, PACS-D16437), Cd-ATPase (J04551), and KdpB (K02670). Vertical lines underneath the conserved sequence motifs indicate their position on the plot.

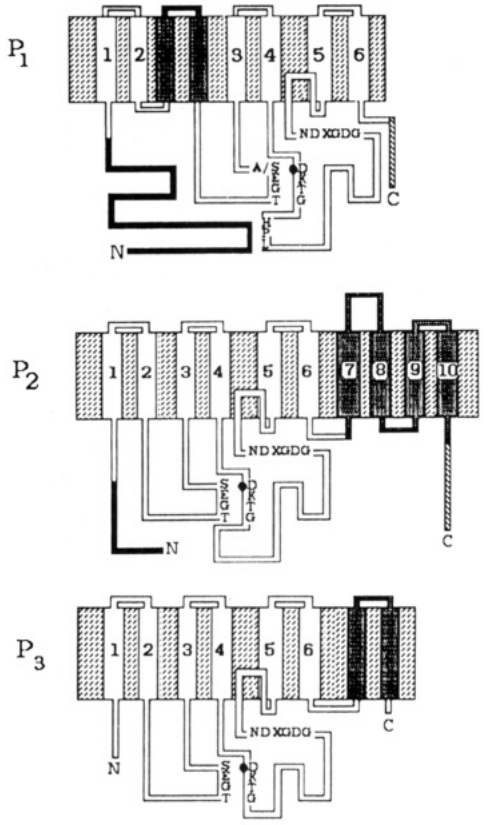

FIGURE 2: Schematic representation of the three major groups of P-type ATPases: heavy-metal-transporting ATPases (P_1), non-heavy-metal-transporting ATPases (P_2), and KdpB (P_3). The core structure is depicted by white blocks, and conserved motifs are identified by the lettering. Gray blocks are for additional and putative transmembrane segments, black lines identify the N-terminus, and hatched blocks show the long C-terminal segment, present in the structure of several but not all of the P-type ATPases in both P_1 and P_2 groups. The core structure of P_1-ATPases is shown incorporating the first two transmembrane segments since in most cases they show higher hydrophobicity than the second pair. The core structure is unaffected if the second pair is selected.

transporting ATPases contain longer and highly charged segments at their N-terminus. Truncation of the N-terminus of the Na,K-ATPase results in a large reduction of the forward rate of the voltage-sensitive Na translocation (Horisberger et al., 1994), changes in the affinity for K at extracellular sites (Vasilets et al., 1993), a 2−3-fold increase in the relative amount of ADP-sensitive phosphoenzyme (Jorgensen, 1994), and an increased rate of K-deocclusion (Wierbicki & Blostein, 1993). Most of these observations are accommodated by the suggestion that in the Na pump the N-terminal segment is required for the effective initiation of the transition from ADP-sensitive to K-sensitive phosphoenzyme, which leads to Na translocation (Jorgensen, 1994). It seems likely that intramolecular interactions in which the N-terminus is engaged are important for closing the access to cation sites from the cytoplasmic portion of the molecule.

The results of these and similar studies on non-heavy-metal-transporting ATPases (P_2) suggest that (i) the N-terminal segment is not important for determining the affinity or specificity for cations of the Ca- or Na,K-ATPases, (ii) the N-terminal segment plays an essential role in the conformational transition associated with cation efflux, and (iii) normal enzyme function requires the interaction between the N-terminal segment and other parts of the ATPase. It is interesting that KdpB, which lacks a long hydrophilic sequence at the N-terminus and can be considered as a naturally truncated P-type ATPase, has a very slow turnover rate. In addition the phosphorylated intermediate of KdpB displays atypical properties: it is almost entirely ADP-sensitive and none of it appears to be discharged by potassium (Naprstek et al., 1992). The central cytoplasmic loop of KdpB, which may be involved in interactions with the N-terminus (see below), is notably shorter than analogous regions of other P-type ATPases (Figure 1).

In striking contrast to the structural diversity of the N-terminal segment of non-heavy-metal ATPases (P_2), all heavy-metal-transporting ATPases (P_1) have a very similar domain of 70 amino acid residues repeated up to six times (Petrukhin et al., 1994). The similarity between the sequence of these repetitive segments and the structure of some mercury-binding proteins was immediately noticed in the first structural analysis of this group of ion pumps, and a role in the binding of heavy-metals was suggested (Silver et al., 1993). In fact, the synthetic peptide GMTCASCVHNIE, which corresponds to the fourth metal-binding repeat of the Wilson's disease protein, has been shown to bind copper cations with 1:1 stoichiometry (Lutsenko et al., 1994). It should be stressed, however, that the cation specificity of almost all heavy-metal-transporting ATPases remains to be characterized. In fact, only for Cd-ATPase have the Cd-stimulated phosphorylation from ATP and transport been directly demonstrated (Tsai & Linet, 1993). For most of the others no similar data are yet available. It remains to be determined, whether the N-terminal domains of P_1-ATPases play a direct role in pumping and providing selectivity and proximate delivery of heavy-metal ions to transport sites or whether their role in cation pumping is less direct. If the former is the case, then the role of the N-terminal domains of heavy-metal-transporting ATPases would be entirely different from non-heavy-metal-transporting ATPases, where cation selectivity is believed to be provided by structures outside the N-terminus. It is possible that a storage function of the N-terminal domain of heavy-metal-transporting ATPases is coupled to the regulation of ATPase activity. In one such mechanism occupancy of the high-affinity heavy-metal-binding sites in this segment could change the interaction between the N-terminal segment and some other parts of the molecule. One candidate for such interactions might be the SEHPL-containing segment immediately following the phosphorylation site in the central loop (Figure 2 and below). Release of an interaction between the N-terminal segment and the SEHPL-containing segment by the binding of a heavy metal ion might activate transphosphorylation from ATP to the enzyme, which in turn would stimulate translocation of the bound ion. A regulatory role of the N-terminal sequence could be a feature shared by all P-type ATPases. For example, in SERCA2 Ca-ATPase phosphorylation of Ser^{38} by Ca/calmodulin-dependendent protein kinase II was shown to increase V_{max} for Ca transport (Toyofuku et al., 1994). Recently Ser and Thr residues in the N-terminal segment of another non-heavy-metal-transporting Na,K-ATPase were identified as a targets of protein kinase C phosphorylation (Beguin et al., 1994).

2. Structurally Diverse Segment after the Phosphorylation Site Is an Essential Element in Cation-Dependent Conformational Transitions. Since the influential work of Jorgensen (1975) on the proteolytic digestion of Na,K-ATPase it has

been clear that the binding of cations causes a large conformational change in the P-type ATPase molecule; exposure to proteolytic attack of the N-terminus of the α-subunit and the central cytoplasmic loop was changed reciprocally upon Na or K binding, hinting that in at least one of the forms of the enzyme these two segments can interact. Recent data indicate that a structurally diverse region located in the central cytoplasmic loop, C-terminal to the phosphorylation site (Figure 2), could be an essential element in the P-type ATPase molecule. Sequence analysis of several putative Cu-transporting ATPases revealed that all of them contain the sequence SEHPL downstream from the phosphorylation site (Tanzi et al., 1993). The crucial role of this segment was highlighted when it was demonstrated that mutation of H to Q in this segment of the Wilson disease gene product resulted in the disease phenotype (overaccumulation of Cu in the liver; Tanzi et al., 1993; Tomas et al., 1995). This particular sequence with invariant His residues is a characteristic feature of P_1-ATPases and is absent in the other two groups of P-type ATPases. Considering the close proximity of the SEHPL sequence to the phosphorylation site and the fact that His residues are often found in heavy-metal binding sites, it may be that binding of the heavy metal in this particular region (or in the interface between this segment and the N-terminal domain) might stimulate cation translocation.

Involvement of the corresponding region of other P-type ATPases in cation-dependent rearrangements has been experimentally demonstrated. In the SR Ca pump, this segment was shown to be the site of interaction with phospholamban (James et al., 1989; Toyofuku et al., 1993). Phospholamban interacts only with Ca-free form of Ca-ATPase, and it is released when the ATPase is converted to the Ca-bound state (James et al., 1989). The analogous region of the plasma membrane Ca-ATPases was shown to bind a synthetic calmodulin-binding domain (Falchetto et al., 1991). The interaction of calmodulin with the plasma membrane Ca-ATPase results in a 20-fold increase in the affinity for Ca (Larsen et al., 1981). These data taken together shed light on a probable role for this region that is just C-terminal to the phosphorylation domain. More experimental evidence is needed to clarify the role of this segment, but it is reasonable to speculate that conformational changes of this site trigger the cation-extrusion steps in the catalytic cycle of the pump. The variations in sequence and size in the region after the phosphorylation site of the P-type ATPases, which transport the same cations may reflect differences in regulatory mechanisms acting on different pumps.

3. C-Terminal Domain Involvement in the Cation-Translocation Structure. One of the most noticeable differences between P_1- and P_2-ATPases is associated with the absence or presence of several additional transmembrane segments in the C-terminal part of the molecule (Figures 1 and 2). The role of the C-terminal part of the non-heavy-metal-transporting ATPase remains a very intriguing question. It is not clear why Cd-ATPase can transport cations without an additional 20−40 kDa segment and the Ca-ATPase cannot. Either the properties and configuration of the cation pathways are entirely different for the two groups of P-type ATPases or the C-terminus is involved in functions other than direct binding of cations.

The resistance of the C-terminus of the Na,K- and H,K-ATPase to proteolytic digestion in the presence of K ions led to the suggestion of the involvement of this region along with other transmembrane segments in cation occlusion (Capasso et al., 1993). However, the replacement of the several charged amino acid residues in the C-terminal part of the Na,K-ATPase by site-directed mutagenesis (Van Huysse et al., 1993; Jewell-Motz & Lingrel, 1993) and chemical modification of Cys^{964} (Nagai et al., 1986) do not have noticeable effects on ATPase activity. Chemical modification of a Glu residue in the tenth transmembrane segment did result in inhibition of Rb occlusion by Na,K-ATPase (Goldshleger et al., 1992); however, the possibility remains that this effect was indirect. Mutation of a Glu residue in M8 (the eighth transmembrane segment of Ca-ATPase) and measurements of Ca transport and occlusion indicate that in the Ca pump this residue is important for overall activity, but it is not involved directly in cation binding (Vilsen, 1993). In fact, data from site-directed mutagenesis of SR Ca-ATPase and mutagenesis and chemical modification of Na,K-ATPase (Vilsen et al., 1989; Clark et al., 1989, 1993; Andersen & Vilson, 1992, 1994; Vilsen, 1992, 1993; Arguello & Kaplan, 1994; Arguello & Lingrel, 1995; Skerjanc et al., 1993) focus our attention on the membrane segments M4, M5, and M6 as being important for cation binding and occlusion rather than on the C-terminal domain, since almost all mutations causing a reduction in the affinity for cations are clustered in these regions. Thus more experimental data are needed in order to decide which, if any, of the C-terminal segments of the P_2-ATPases participate directly in cation binding and transport.

A different (or additional) role for the C-terminal domain can be envisaged on the basis of the results of studies of membrane topology and insertion. It has been noted that the functionally important amphipathic M5M6 hairpin which follows the ATP binding loop cannot by itself be incorporated appropriately into membranes (Homareda et al., 1993; Bamberg & Sachs, 1994). Recent studies of the gastric H,K-ATPase (Bamberg & Sachs, 1994) suggest that the C-terminal domain may play a critical role in assisting the membrane insertion of the M5M6 hairpin. Spontaneous release of the M5M6 hairpin from the posttryptic membrane preparation into the aqueous phase (see below) is also accompanied by reorganization of the C-terminal domain of the α-subunit of Na,K-ATPase (Lutsenko et al., 1995). This points to the possible role of the C-terminal domain in support of the proper folding of the M5M6 hairpin. Several charged residues (about five) and proline residues (four to five) in the region containing the M5M6 hairpin of P_2-ATPases make its presence in the membrane energetically unfavorable, while the corresponding hairpin of P_1-ATPases has no charged residues and has fewer helix-breaking residues (two prolines, Figure 3). Cd-ATPase represents an interesting exception among P_1-ATPases: the conserved Met in putative M6 segment is replaced by Asp, while invariant Pro in M5 is replaced by Lys. The absence or presence of an extended C-terminal segment in the P_1- and P_2-ATPases, respectively, is apparently correlated with the potential intramembrane stability of the M5M6 hairpins.

4. Transmembrane Hairpin Following the ATP-Binding Domain Plays a Key Role in Coupling of ATP Hydrolysis and Transport of the Cations. We have recently proposed a key role in cation transport and coupling for the region of

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CopB          KMIQNLWWGA GYNIIAIPLA AGILAPIGLI LSPAVGAVLM SLSTVVVALN ALTLK-
CopA          KQ--NLFWAF IYNTIGIPFA AFGF-------LNPIIAGGAM AFSSISVLLN SLSLNR
Cu-yeast      KL--NLFWAL CYNIFMIPIA MGVLIPWGIT LPPMLAGLAM GFSSVSVVLS SLMLK-
Cu-human/WD   RI--NLVLAL IYNLVGIPIA AGVFMPIGIV LQPWMGSAAM AASSVSVVLS SLQLK-
Cu-human/MNK  RI--NFVFAL IYNLVGIPIA AGVFMPIGLV LQPWMGSAAM AASSVSVVLS SLFLK-
Cd-ATPase       TLNIIKAN ITFAIGIKII ALLLVIPGW- LTLWIAILSD MGATILVALN SLR-
Consensus       --NL..A. IYN..GIPIA AG...P.G.. L.P.....AM ..SSVSV.L. SL.LK-

                          M5                              M6
Ca-ATPase SR    SNVGEVVCIFLT AALGLPEALI PVQLLWVNLV TDGLPATALG FNPPDLD-IM DR
Ca-ATPase PM    VNVVAVIVAFTG ACITQDSPLK AVQMLWVNLI MDTLASLALA TEPPTESLLL GK
Na,K-ATPase     SNIPEITPFLIF IIANIPLPLG TVTILCIDLG TDMVPAISLA YEQAESDIMK
H,K-ATPase      KNIPELTPYLIY ITVSVPLPLG CITILFIELC TDIFPSVSLA YEKAESDIMHLR
Consensus        N..E.....  .....P.PL. .V..L...L. TD..P...LA .E....D...
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FIGURE 3: Alignments of the M5M6-containing region of P_1 (upper) and P_2 (lower) ATPases. The sequences of transmembrane hairpins following the conserved MXGDGXNDXP sequence were aligned. The most hydrophobic stretches are underlined, but these do not necessarily correspond precisely to the intramembrane segments.

the Na,K-ATPase immediately after the ATP binding domain, which includes the M5M6 hydrophobic hairpin. In posttryptic preparations the M5M6 hairpin spontaneously leaves the membrane following removal of the occluded cations, and can be collected in the supernatant. This quite unexpected behavior for transmembrane peptides is specific for the M5M6 hairpin and makes it likely that a direct interaction with transported cations is involved (Lutsenko et al., 1995). Site-directed mutagenesis of SR Ca-ATPase and Na,K-ATPase and chemical modification of Glu^{779} identified this hairpin as being important for cation (Ca, K, and Na) binding and occlusion. It is quite possible that segments M5M6 and M4 provide most of the residues for selective caging and release of cations in the intramembrane part of P-type ATPases (as suggested for Ca-ATPase; Andersen & Vilsen, 1994). The sequence of these segments varies among P-type ATPases; however, a good correlation (Figure 3) between the structure and type of transported cation can be seen, especially for transmembrane segment M6.

The precise way in which the M5M6 hairpin is involved in coupling and transport is not known. Two obvious possibilities are (i) that the hairpin contains parts of the occlusion cavity for monovalent cations and the transport involves piston-like movements of this segment or (ii) that cation binding by the M5M6 transmembrane helices stabilizes energeticaly unfavorable intrahelix interactions that form the basis of rapid conformational transitions (twisting or rocking of the helices involved). Absence of C-terminal transmembrane segments from the structure of heavy-metal-transporting ATPases indicates that most likely in these ATPases at least part of the M5M6 hairpin interacts with the lipids, while in non-heavy-metal ATPases this segment could be completely screened from the lipids by other protein segments. The best candidate for the "moving" part of the machinery is probably the N-terminal portion of the M5M6 transmembrane hairpin (Figure 2). Our recent data indicate that this segment is resistant to proteolytic digestion in the presence of occluded K, but can be easily cleaved off, when the Na pump is phosphorylated (Lutsenko et al., 1994, 1995). Moreover, the N-terminal segment of the M5M6 hairpin is connected to the hinge region, a highly conserved segment in the structure of all P-type ATPases, which includes two invariant aspartic residues. These residues (Asp^{714} and Asp^{710} in the structure of pig Na,K-ATPase) were found to be labeled by γ-4-(*N*-2-chloroethyl-*N*-methylamino) benzylamide ATP (ClR-ATP), an ATP analog, in a conformation-dependent fashion (Ovchinnikov et al., 1987). Furthermore, the replacement of a Tyr residue in the N-terminal segment of the M5M6 hairpin of the SR Ca-ATPase results in uncoupling of Ca transport and ATP hydrolysis (Andersen, 1995). These data emphasize the close proximity of the phosphorylation and ATP-binding domains to the intramembrane region, essential for cation binding. It seems clear that, independent of the precise role of the M5M6 domain, the N-terminal part of this segment is an important part of the cation-translocation machinery.

In summary, we propose that the various P-type ATPases can be grouped according to their major structural features which correlate with the type of transported cation. All P-type ATPases are based upon a common core, which includes an ATP-binding domain, a cationic transport pathway, and loops that link these two functions. Specific requirements for different cations and the regulation of their transport are provided by structurally diverse additional segments.

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