Phenotypic Screening of Mutations in Pmr1, the Yeast Secretory Pathway Ca$^{2+}$/Mn$^{2+}$-ATPase, Reveals Residues Critical for Ion Selectivity and Transport*

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Thirty-five mutations were generated in the yeast secretory pathway/Golgi ion pump, Pmr1, targeting oxygen-containing side chains within the predicted transmembrane segments M4, M5, M6, M7, and M8, likely to be involved in coordination of Ca$^{2+}$ and Mn$^{2+}$ ions. Mutants were expressed in low copy number in a yeast strain devoid of endogenous Ca$^{2+}$ pumps and screened for loss of Ca$^{2+}$ and Mn$^{2+}$ transport on the basis of hypersensitivity to 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) and Mn$^{2+}$ toxicity, respectively. Three classes of mutants were found: mutants indistinguishable from wild type (Class 1), mutants indistinguishable from the pmr1 null strain (Class 2), and mutants with differential sensitivity to BAPTA and Mn$^{2+}$ toxicity (Class 3). We show that Class 1 mutants retain normal/near normal properties, including $^{40}$Ca transport, Golgi localization, and polypeptide conformation. In contrast, Class 2 mutants lacked any detectable $^{40}$Ca transport; of these, a subset also showed defects in trafficking and protein folding, indicative of structural problems. Two residues identified as Class 2 mutants in this screen, Asn$^{774}$ and Asp$^{778}$ in M6, also play critical roles in related ion pumps and are therefore likely to be common architectural components of the cation-binding site. Class 3 mutants appear to have altered selectivity for Ca$^{2+}$ and Mn$^{2+}$ ions, as exemplified by mutant Q$^{783}$A in M6. These results demonstrate the utility of phenotypic screening in the identification of residues critical for ion transport and selectivity in cation pumps.

Ion pumps belonging to the family of P-type ATPases occur in all cells, where they drive transmembrane ion gradients of up to 10,000-fold (reviewed in Ref. 1). Well known members of the family include the Na$^{+}$/K$^{+}$-ATPase of animal cells, H$^{+}$/ATPase of fungi and plants, and various Ca$^{2+}$-ATPases found in plasma membrane and endomembrane compartiments. Individual pumps have evolved distinct cation selectivities to fulfill a variety of different physiological functions including Ca$^{2+}$ homeostasis, acid (H$^{+}$) extrusion, generation of Na$^{+}$ and K$^{+}$ electrochemical gradients, and the detoxification of soft metals (Cu$^{2+}$, Cd$^{2+}$, and Zn$^{2+}$). Despite their differences in cation selectivity, P-type ATPases share many structural and mechanistic similarities. Remarkably, the electron micrograph structures (8 Å) of the fungal H$^{+}$/ATPase and the sarcoplasmic reticulum Ca$^{2+}$/ATPase are virtually superimposable within the dimensions of the membrane (2, 3). In both cases, densities corresponding to ten transmembrane helices are clearly visible, of which three are clustered to enclose a well defined pore, plausibly the pathway for ion translocation, extending from the cytoplasmic to the luminal/extracellular space. A variety of experimental evidence points to three helices, transmembrane segments M4, M5, and M6, that are most likely to be involved in transport. For example, in the sarcoplasmic reticulum Ca$^{2+}$/ATPase, mutagenesis of residues in M4, M5, and M6 prevent cation binding and inactivate transport (reviewed in Ref. 4), and cysteine residues engineered into M4 and M6 can be sulfide-linked (5). The proximity of M5 and M6 may be inferred from studies on the fungal H$^{+}$/ATPase showing the presence of a salt bridge between residues in these two helices (6).

Defining the residues that compose the ion-binding site(s) is a first step toward understanding how vectorial transport occurs. Extensive mutagenesis studies have contributed to the emerging molecular picture of the transport site in which oxygen-containing side chains coordinate one or more cations. Thus, in SERCA, residues Glu$^{509}$ (M4), Glu$^{771}$ (M5), and Asn$^{796}$, Thr$^{799}$, and Asp$^{806}$ (M6), are required for binding two Ca$^{2+}$ ions (reviewed in Ref. 4). Similar studies in the Na$^{+}$/K$^{+}$-ATPase have revealed the important role of residues Ser$^{775}$ (M5), Asp$^{804}$, and Asp$^{806}$ (M6) in K$^{+}$ and possibly Na$^{+}$ binding (reviewed in Ref. 7). Interestingly, Asn$^{796}$ and Asp$^{806}$ of SERCA occupy equivalent positions to Asp$^{804}$ and Asp$^{806}$ in Na$^{+}$/K$^{+}$-ATPase, suggesting that the architecture of the ion-binding site may be similar in the two enzymes. Additional studies on other ion pumps will be key to determining whether this similarity extends throughout the family.

The P-type ATPases offer a striking paradigm for the evolutionary development of ion selectivity; yet the molecular basis for selectivity remains one of the fundamental unanswered questions in the field. From studies on other classes of transport proteins, it is clear that selectivity is determined by the local environment around key residues (8, 9). However, it is difficult to identify, from conventional site-directed mutagenesis alone, which residues are important for distinguishing between different ions, such as Ca$^{2+}$ and Na$^{+}$. Despite having completely nonoverlapping ion selectivities, four of five residues important for Ca$^{2+}$ binding in SERCA are conserved in equivalent positions in the Na$^{+}$/K$^{+}$-ATPase sequence so that these residues cannot be considered to define ion selectivity.

Here we report on the mutagenesis of every oxygen-containing side chain within membrane segments M4–M8 of Pmr1, the yeast secretory pathway/Golgi ion pump. Previous studies...
from our lab (10, 11), as well as others (12–14), have suggested that Pmr1 mediates the high affinity transport of Ca\(^{2+}\) and Mn\(^{2+}\) into the secretory pathway for a variety of secretory functions, including protein sorting, processing, and glycosylation. A novel aspect of this study is the phenotypic screening of yeast mutants to identify residues that are critical for Ca\(^{2+}\) and Mn\(^{2+}\) transport and selectivity. We show that this approach greatly simplifies the analysis of mutants and can be used to screen large numbers of mutants in future random mutagenesis studies. We expect that this approach can be extended to the study of heterologous ion pumps expressed in yeast, including Ca\(^{2+}\)-ATPases from animal and plant systems (15, 16).

**EXPERIMENTAL PROCEDURES**

**Media, Strains, and Growth Assays—** Yeast strains were grown in defined media containing yeast nitrogen base (6.7 g/liter; Difco), dextrose (2%), and supplements as needed. **PMR1**-containing plasmids were introduced into strain K616 (17), which carries null alleles of calcineurin B (CNB1) and two Ca\(^{2+}\)-ATPases (PMr1 and PMC1), resulting in low basal Ca\(^{2+}\) pump activity, as described previously (10). Growth assays were performed by inoculating 100 μl of YNB medium in a 96-well plate with 5-μl of a saturated seed culture followed by incubation for 48 h at 30 °C. Where indicated, MnCl\(_2\) or BAPTA\(^1\) was added to the medium prior to inoculation, and the pH was adjusted to 6.0 with NaOH. Cultures were thoroughly mixed by gentle vortexing, and growth was monitored by measuring absorbance at 600 nm in a SPECTRAmax 340 microplate reader (Molecular Devices). Relative growth was expressed as the fraction of the absorbance of the control culture (no additions).

**Plasmids and Mutagenesis—** YEpHR1, a yeast 2μ plasmid carrying the **PMR1** coding sequence under control of a tandem repeat of a yeast endogenous promoter (not shown). Each mutant was screened for sensitivity to BAPTA and Mn\(^{2+}\), indicating that the two ions can play largely surrogate roles in supporting yeast growth. Because standard yeast media have nearly a 100-fold excess of Ca\(^{2+}\) relative to Mn\(^{2+}\), and the latter is efficiently removed at low chelator concentrations, the observed growth inhibition by BAPTA in **pmr1** mutants represents a titration of the remaining Ca\(^{2+}\). Hypersensitivity of **pmr1** mutants to Mn\(^{2+}\) toxicity is a specific consequence of loss of Mn\(^{2+}\) transport. Although essential for growth, excess Mn\(^{2+}\) is toxic and must be removed by delivery into the secretory pathway by Pmr1 and subsequent exit from the cell. Thus, we reasoned that sensitivity to BAPTA and Mn\(^{2+}\) toxicity would be a preliminary indication of the Ca\(^{2+}\) and Mn\(^{2+}\) transporting activity of Pmr1 mutants.

35 mutations were made at potential cation-binding sites (Asp, Glu, Asn, Gln, Ser, Thr, and Tyr) in the predicted transmembrane segments M4, M5, M6, M7, and M8 of Pmr1. Residues were substituted with Ala, and in the case of acidic residues, also as follows: Asp → Asn, Glu and Glu → Asp, Gln. In one case, an Asp was introduced to replace Ala\(^{749}\) in M5 so as to mimic the sequence of the SERCA pump. Pmr1 mutants were introduced into the yeast strain K616 (Δpmr1Δpmc1Δcnb1), which we have previously shown to be devoid of endogenous Ca\(^{2+}\) pump activity (10). To ensure low level expression of the pump for phenotype analysis, mutants were expressed under control of the heat shock element, from single copy/CEN plasmids, and grown at 30 °C. We found that in the absence of heat shock, leak-through expression of Pmr1 was similar to native levels of expression from the endogenous promotor (not shown). Each mutant was screened for sensitivity to BAPTA and Mn\(^{2+}\) in quantitative liquid growth assays, as described under "Experimental Procedures." Plasmids containing wild type **PMR1** (YCpHR1) and the empty vector (YCpH2) were also transformed into strain K616 as controls.

Mutants fell into one of three classes, as exemplified in Fig. 1. Class 1 mutants, such as mutant T826A, were indistinguishable from wild type in both BAPTA and Mn\(^{2+}\) tolerance assays and were assumed to have largely normal transport function. Class 2 mutants, such as mutant D778A, displayed a null phenotype for both BAPTA and Mn\(^{2+}\) tolerance, suggesting a complete loss of function. Interestingly, some mutants displayed differential tolerance to BAPTA and Mn\(^{2+}\) and were grouped as Class 3 mutants. An extreme example is mutant Q783A (Fig. 1), in which tolerance to BAPTA is at wild type levels, whereas tolerance to Mn\(^{2+}\) is similar to the null mutant, suggesting that Ca\(^{2+}\) transport is retained but Mn\(^{2+}\) transport is lost. Two other mutants (E329A and A749D) showed intermediate profiles of BAPTA tolerance but complete loss of Mn\(^{2+}\) tolerance. These are putative ion selectivity mutants.

A complete classification of all 35 mutants reported in this study is listed in Table I. None of the residues targeted for mutagenesis in membrane segments M4 and M8 were found to be essential for function; although the apparently conservative substitutions E329D (M4) and D856E (M8) were Class 2 (loss-of-function) mutants, substitution with Ala restored function either partially (E329A) or completely (D856A). Alanine sub-

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1 The abbreviations used are: BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N',N,N'-tetraacetic acid; PAGE, polyacrylamide gel electrophoresis; SERCA, sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase; ER, endoplasmic reticulum.
Substitutions of residues Gln742 (M5), Asn774 and Asp778 (M6), and Thr817 (M7) resulted in loss-of-function phenotypes. Additional substitution of Asp778 with Glu and Asn also failed to restore function, confirming the importance of this residue in cation transport.

Biogenesis, Targeting, and Ion Transport Characteristics of

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 1.** Representative examples of three classes of pmr1 mutants. Host strain K616 (Δpmr1Δpmc1Δcnb1) was transformed with a low copy vector (YCpH1) carrying either wild type PMR1 (WT) or mutants T826A, D778A, and Q783A. Cells were grown in YNB medium supplemented with BAPTA (A) or Mn2+ (B) as indicated for 48 h at 30 °C. Growth (A600) was normalized to control (no additions). T826A was representative of Class 1 mutants and resembled the wild type in both growth assays. D778A, a Class 2 mutant, displayed a hypersensitivity to growth inhibition by BAPTA and Mn2+, similar to the null strain (vector). The Class 3 mutant Q783A had differential sensitivity to BAPTA and Mn2+, suggestive of a change in ion selectivity. □, WT; ○, vector; ●, T826A-Class 1; ■, D778A-Class 2; ▲, Q783A-Class 3.

| Mutation | Membrane span | Phenotype classa | ATP-protectible trypsinolysisb | Localizationc | Golgi | ER |
|----------|---------------|------------------|-------------------------------|---------------|-------|----|
| S319A    | M4            | x                |                 Yes         |               |       |    |
| S321A    | M4            | x                |                 No          |               |       |    |
| E329A    | M4            | x                |                 x           |               |       |    |
| E329D    | M4            | x                |                 x           |               |       |    |
| E329Q    | M4            | x                |                 x           |               |       |    |
| T740A    | M5            | x                |                 x           |               |       |    |
| Q742A    | M5            | x                |                 x           |               |       |    |
| S744A    | M5            | x                |                 x           |               |       |    |
| T745A    | M5            | x                |                 x           |               |       |    |
| S746A    | M5            | x                |                 x           |               |       |    |
| A749D    | M5            | x                |                 x           |               |       |    |
| S751A    | M5            | x                |                 x           |               |       |    |
| S756A    | M5            | x                |                 x           |               |       |    |
| T757A    | M5            | x                |                 x           |               |       |    |
| Q769A    | M6            | x                |                 x           |               |       |    |
| N774A    | M6            | x                |                 x           |               |       |    |
| D778A    | M6            | x                |                 x           |               |       |    |
| D778N    | M6            | x                |                 x           |               |       |    |
| D778E    | M6            | x                |                 x           |               |       |    |
| Q783A    | M6            | x                |                 x           |               |       |    |
| S784A    | M6            | x                |                 x           |               |       |    |
| T808A    | M7            | x                |                 x           |               |       |    |
| D810A    | M7            | x                |                 x           |               |       |    |
| T817A    | M7            | x                |                 x           |               |       |    |
| T819A    | M7            | x                |                 x           |               |       |    |
| T826A    | M7            | x                |                 x           |               |       |    |
| Y828A    | M7            | x                |                 x           |               |       |    |
| T845A    | M8            | x                |                 x           |               |       |    |
| T846A    | M8            | x                |                 x           |               |       |    |
| T848A    | M8            | x                |                 x           |               |       |    |
| T850A    | M8            | x                |                 x           |               |       |    |
| D856A    | M8            | x                |                 x           |               |       |    |
| D856N    | M8            | x                |                 x           |               |       |    |
| D856E    | M8            | x                |                 x           |               |       |    |
| N859A    | M8            | x                |                 x           |               |       |    |

* Growth inhibition by BAPTA and Mn2+ was evaluated from low copy expression of mutants in the pmr1 null strain as described in the text. Mutants fell into three classes: wild type phenotype for both BAPTA and Mn2+ (Class 1), null phenotype for both BAPTA and Mn2+ (Class 2), and differential response to BAPTA and Mn2+ (Class 3).

* Membranes were treated with trypsin in the presence or absence of 5 mM ATP, and the presence of an ATP-protectible 52-kDa polypeptide, as described in the text, is indicated.

* Yeast lysates from cultures grown at 30 °C were fractionated on sucrose density gradients as described under "Experimental Procedures," and 20-μg aliquots of individual fractions were subjected to SDS-PAGE and Western Blotting with anti-Pmr1 antibody. Nonoverlapping distribution of Pmr1 to Golgi (26–34% sucrose) or endoplasmic reticulum (42–54% sucrose) was determined on the basis of colocalization with organellar markers (10).
pmr1 Mutants—As the first step in a systematic biochemical analysis of mutants, we checked expression levels in rapid, small-scale, total membrane preparations of yeast transformants, following heat shock-induced expression from multicopy (2μ) plasmids (10). Western blots using anti-Pmr1 polyclonal antibody provided evidence for the biogenesis and membrane insertion of all mutant Pmr1 proteins, albeit with some variation in expression levels, as shown in the samples in Fig. 2. Curiously, Class 2 mutants N774A, D778E, T817A, and D856E had distinctly faster mobility on SDS-PAGE (Figs. 2 and 4). Mutant plasmids were rescued from the transformed yeast strains and analyzed by restriction enzyme mapping and DNA sequencing to confirm the integrity of the mutant gene (not shown). The faster mobility appeared not to be related to altered glycosylation or phosphorylation of the protein2 and was tentatively assumed to be a first indication of abnormal conformation.

 Trafficking of mutant Pmr1 proteins was examined on well defined sucrose density gradient fractionations of yeast lysates. This analysis was essential to normalize ion transport activity to Pmr1 expression levels in the Golgi fractions and can be considered a second indication of structural problems, because incorrectly folded ATPases are retained and subsequently degraded in the yeast endoplasmic reticulum (21). Fig. 3 shows the subcellular localization of a subset of Pmr1 mutants; a complete listing of organelar localization is found in Table I. We have previously demonstrated a clear separation of the lighter Golgi fractions (fractions 4–6 in Fig. 3) from endoplasmic reticulum, the latter being distributed in the denser half of the sucrose gradient (fractions 8–11). All of the Class 1 mutants showed normal trafficking to the Golgi, consistent with their normal phenotype. Among the other two classes of mutants, some displayed normal trafficking, as depicted in Fig. 3 for D778N. Others were retained in the endoplasmic reticulum, either partially (A749D) or completely (D778E). When the target residue was acidic (Asp778 and Asp856), there was a striking difference in targeting depending upon the nature of the substitution. Surprisingly, substitution with another acidic residue (Asp → Glu and Glu → Asp) led to ER retention and concomitant loss of function (Table I), whereas substitution with a neutral residue allowed correct Golgi targeting. Another interesting finding from this study was that complete ER retention consistently correlated with faster mobility of the mutant Pmr1 polypeptide upon SDS gel electrophoresis, possibly indicating an abnormal protein conformation that was resistant to unfolding in SDS. It should be noted that mutants showing ER retention continued to show trafficking problems under normal culture temperatures of 30 °C, which obviated the possibly deleterious effects of heat shock-induced expression (at 37 °C) on protein targeting.

 Individual fractions of the sucrose gradient were assayed for 45Ca transport activity. As described earlier, the host strain was essentially devoid of endogenous Ca2+ pump activity,

2 D. M. Mandal and R. Rao, unpublished results.
efficiency of ATP protection against proteolysis for all mutants in this study.

Manganese Inhibition of Calcium Transport Is a Measure of Mn$^{2+}$ Selectivity—We have observed saturatable $^{54}$Mn uptake into Golgi vesicles isolated from strains expressing wild type Pmr1 but not from the null mutant K616, consistent with the proposed role of Pmr1 in Mn$^{2+}$ transport. An alternative approach is to use Mn$^{2+}$ inhibition of $^{45}$Ca transport to assess Mn$^{2+}$ transport activity, as reported earlier (11). Here, we examine the effectiveness of Mn$^{2+}$ inhibition in the putative ion selectivity (Class 3) mutant Q783A. Fig. 5 shows that Mn$^{2+}$ is a potent inhibitor of $^{45}$Ca transport in Golgi vesicles containing wild type Pmr1, with half-maximal inhibition occurring at 0.2 $\mu$M, under conditions where the Ca$^{2+}$ concentration is nominally 0.8 $\mu$M (approximately 10-fold in excess over the $K_{m}$ for Ca$^{2+}$; Refs. 10, and 11). These data suggest that ion selectivity of wild type Pmr1 is Mn$^{2+}$ > Ca$^{2+}$. In contrast, half-maximal inhibition for the Q783A mutant occurred at 12 $\mu$M, a 60-fold shift relative to wild type. These results are consistent with a dramatic reduction in the affinity for Mn$^{2+}$, and consequently hypersensitivity to Mn$^{2+}$ toxicity (Fig. 1), in this mutant.

**DISCUSSION**

In this study, we describe an effective strategy to screen for amino acid residues critical for ion transport and selectivity in yeast mutants of the secretory pathway/Golgi ion pump, Pmr1. As a test of this approach, we used alanine-scanning site-directed mutagenesis of acidic and polar residues within the transmembrane segments most likely to constitute the translocation pore. Mutants were readily classified in one of three groups based upon cell growth in BAPTA-$\cdot$supplemented medium. Class 1 mutants did not display hypersensitivity to either BAPTA or Mn$^{2+}$, a first indication of normal function. Further analysis confirmed that these mutants showed normal trafficking to the Golgi, had significant $^{45}$Ca transport activity and appeared to be correctly folded based upon the ability of ATP to protect against trypsinolysis. Most of the residues targeted for mutagenesis in this study fell into this class (Fig. 6) and were not essential for pump function, as was observed in previous site-directed mutagenesis studies of this scale on the Na$^{+}$/K$^{+}$-ATPase, H$^{+}$-ATPase, and SERCA (reviewed in Refs. 4, 7, and 22).

Class 2 mutants were hypersensitive to both BAPTA and Mn$^{2+}$ toxicity, indicative of a complete lack of pump function, which was confirmed by the lack of $^{45}$Ca transport activity in isolated Golgi fractions. To distinguish between structural and functional derangements, we analyzed protein trafficking and sensitivity to trypsinolysis. Mutants that showed normal Golgi trafficking also displayed ATP-protectable trypsin fragments, whereas those that showed ER retention failed to generate ATP-protectable tryptic peptides. The latter also displayed increased mobility upon gel electrophoresis, for reasons that are currently unresolved. These observations are consistent with abnormal polypeptide folding and indicate sites that are sensitive to structural perturbations. Unexpectedly, Asp $\rightarrow$ Glu and Glu $\rightarrow$ Asp substitutions caused structural problems, whereas replacement with the neutral alanine did not. Although the perturbation caused by introduction of the longer glutamate side chain may be rationalized as a steric effect, it is not clear why substitution of aspartate, a shorter carboxylate side chain, in place of glutamate was more deleterious than that of alanine. One possibility is that within the confines of the membrane, differences in dihedral angle preferences for the carboxyl group of aspartate and glutamate may be important.

Fig. 6 summarizes the results of our study. None of the target residues in transmembrane segments M4 or M8 were essential for function, although substitution-specific loss of function was observed at Glu$^{329}$ and Asp$^{356}$. Structural and trafficking defects were also seen with alanine substitution of Gln$^{742}$ (M5), Asn$^{774}$ (M6), and Thr$^{817}$ (M7), resulting in complete loss of function. However, alanine and asparagine substitution mutants of Asp$^{778}$ (M6) showed normal trafficking and polypeptide conformation yet lacked transport activity, making this residue a good candidate for the cation-binding pocket. Strikingly similar findings have been reported for mutations at residues

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3 Y. Wei and R. Rao, unpublished results.
equivale to Asn774 and Asp778 of the plasma membrane Ca$$^{2+}$$-ATPase: thus, mutant N879A was retained in endoplasmic reticulum, whereas mutant D883A showed normal trafficking to the plasma membrane (23). Both showed a complete loss of Ca$$^{2+}$$ transport activity (23, 24). Alanine substitutions of the equivalent residues in the Na$$^{+}$$/K$$^{+}$$-ATPase, Asp804 and Asp808, however, do not prevent trafficking to the plasma membrane, as judged by ouabain binding ability in intact cells (7), but are also associated with loss of transport activity. Thus, these two sites appear to be common components of the cation-binding site in most P-type ATPases. One exception is the fungal H$$^{+}$$-ATPase, Pma1, where Asp$$^{390}$$ (equivalent to Asp$$^{778}$$ of Pmr1) forms a salt bridge with Arg$$^{695}$$ in M5; simultaneous substitution of both residues with alanine does not alter function (6).

The simple growth screens based on BAPTA and Mn$$^{2+}$$ toxicity in pmr1 mutants are capable of distinguishing between essential and nonessential residues and can be used to screen large numbers of randomly generated mutants in future studies. We also show that the screens can identify potential sites of cation selectivity. Three such sites were identified in this study; in each case, the mutants showed greater sensitivity to Mn$$^{2+}$$ toxicity than to that of the Ca$$^{2+}$$ chelator BAPTA in growth assays, suggesting that the mutation had a more deleterious effect on Mn$$^{2+}$$ transport. However, in a previous study, we have reported on a mutation with the converse effect; D53A, within the N-terminal modulatory EF hand-like domain in Pmr1, was indistinguishable from wild type in the Mn$$^{2+}$$ toxicity assay (11), but BAPTA toxicity in this mutant was intermediate between wild type and the pmr1 null mutant. We showed that apparent Mn$$^{2+}$$ affinity was slightly increased relative to wild type, whereas there was a 11-fold reduction in Ca$$^{2+}$$ transport affinity in this mutant (11). Here, we show that Q783A has normal Ca$$^{2+}$$ transport but a 60-fold reduction in the apparent affinity for Mn$$^{2+}$$. Only one such mutation with a similar dramatic effect on ion selectivity has been reported in studies on other ion pumps; substitution of Ser$$^{770}$$ in M5 with alanine in the Na$$^{+}$$/K$$^{+}$$-ATPase causes a 30-fold decrease in only K$$^{+}$$. It is worth noting that only two of five residues required for Ca$$^{2+}$$ binding in SERCA were essential in Pmr1. A likely interpretation is that unlike SERCA, which has a stoichiometry of two Ca$$^{2+}$$ transported per ATP, Pmr1 transports only one cation transported per ATP. In the sequence of Pmr1 and its homologues, Ala and Met occupy positions equivalent to Glu$$^{771}$$ (M5) and Thr$$^{770}$$ (M6) of SERCA, with a fewer number of cation-binding sites. These differences support the classification of the newly emergent secretory pathway Ca$$^{2+}$$-ATPases as a group distinct from but related to the well-known endoplasmic reticulum pumps.

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