Minireview

The Yeast Pma1 Proton Pump: a Model for Understanding the Biogenesis of Plasma Membrane Proteins*

Published, JBC Papers in Press, June 12, 2001, DOI 10.1074/jbc.R100022200

Thierry Ferreira‡, A. Brett Mason, and Carolyn W. Slayman§

From the Departments of Genetics and Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut 06510

The Pma1 H\(^+\)-ATPase of Saccharomyces cerevisiae, which functions physiologically to pump protons out of the cell, is one of the most abundant proteins in the yeast plasma membrane (1). It is a 100-kDa polypeptide, anchored in the membrane by 10 hydrophobic \( \alpha \)-helices (Fig. 1) (2) and belonging to a widespread family of cation transporters known as the \( \text{P}_2 \)-type ATPases (6). Members of the \( \text{P}_2 \) family in animal cells include the plasma membrane Na\(^+\),K\(^+\)- and Ca\(^2+\)-ATPases, gastric mucosal H\(^+\), K\(^+\)-ATPase, and sarcoplasmic reticulum Ca\(^2+\)-ATPase.

In recent years, the yeast H\(^+\)-ATPase has emerged as a valuable prototype for studies of plasma membrane biogenesis. Several complementary approaches have been taken, all drawing on the power of yeast genetics. (i) Strains with temperature-sensitive blocks at successive steps in the secretory pathway have made it possible to map the route by which the H\(^+\)-ATPase travels to the plasma membrane (7–9). (ii) Point mutations in the \( \text{PMA1} \) gene have given insights into the structural requirements for proper folding and trafficking of the H\(^+\)-ATPase (10–13). (iii) Suppressors and enhancers of biogenesis-defective \( \text{pma1} \) mutants have revealed new components of the secretory process (14–18). (iv) Finally, by screening for mutations that exacerbate a temperature-sensitive defect in one of the standard COPII coat subunits, a specialized coat protein has been identified that helps to mediate the exit of newly synthesized H\(^+\)-ATPase from the ER (19, 20). In the following sections, recent results from all four approaches are woven together into a stepwise description of H\(^+\)-ATPase biogenesis. A comprehensive review of earlier work can be found in a chapter by de Kerchove d’Exaerde et al. (21).

**H\(^+\)**-ATPase Is Made in Rough ER and Delivered to Plasma Membrane via Secretory Pathway

As expected, Pma1 H\(^+\)-ATPase is synthesized and integrated into the membrane in the rough endoplasmic reticulum. Pulse-chase experiments suggest that it achieves a fully folded structure very rapidly, because it can be protected against trypsinolysis by physiological concentrations of ligands even at the earliest time points (22). The ATPase then travels to the cell surface via the secretory pathway (Fig. 2), as shown by the fact that its biogenesis can be blocked by temperature-sensitive mutations in genes governing successive steps of the pathway: \( \text{SEC18} \) (ER to Golgi), \( \text{SEC7} \) (Golgi to secretory vesicles), and \( \text{SEC6} \) (secretory vesicles to plasma membrane) (8, 9). Interestingly, the 100-kDa H\(^+\)-ATPase undergoes post-translational phosphorylation on multiple Ser and Thr residues during its transit from the ER to the cell surface (9). The functional reason for these stepwise phosphorylations is unknown, although there is good evidence that the last one, occurring at or near the plasma membrane, plays a role in the activation of the ATPase by glucose (9).

The oligomeric state of the mature H\(^+\)-ATPase is not yet certain. Monomers of the closely related Neurospora crassa enzyme are fully active after reconstitution into proteoliposomes (23), but radiation inactivation experiments give a target size of 230 kDa, consistent with a functional dimer (24). Hexameric complexes are recovered on glycerol or sucrose gradients after detergent solubilization (25) and have made it possible to produce two-dimensional crystals for structural studies (2), but there is no clear evidence that such complexes exist in vivo.

**Misfolded H\(^+\)**-ATPase Mutants Serve as Markers for Specialized ER Subdomains

Even though the ER is not as clearly differentiated in yeast as in many mammalian cells, immunofluorescence and immunoelectron microscopy have revealed two morphologically distinct parts: (i) prominent perinuclear elements, continuous with the outer nuclear membrane, and (ii) peripheral tubules, extending outward through the cytoplasm and concentrated in the region immediately beneath the plasma membrane (26). Both are studded with ribosomes and can be labeled by antibodies against ER markers, and it seems likely that they form a single interconnected network (27). If so, one would like to learn where specific plasma membrane proteins are synthesized within the network and where they are packaged into vesicles for shipment to the Golgi. It would also be useful to know where and how such proteins are screened for proper folding before being allowed to leave the ER.

Partial answers to both questions have come from the use of Pma1 H\(^+\)-ATPase as a model plasma membrane protein. Of nearly 300 site-directed mutations that have been introduced throughout the ATPase, amino acid substitutions at 45 positions have led to defects in biogenesis (Fig. 1) (reviewed in Ref. 1). Most (and perhaps all) of these polypeptides are poorly folded, as evidenced by their abnormal sensitivity to trypsin (12, 13). Confocal and immunoelectron microscopy have shown that such mutations trigger a dramatic proliferation of ER-derived membranes, in which misfolded H\(^+\)-ATPase accumulates along with standard ER markers such as Kar2p (10, 11, 13, 21). When wild-type H\(^+\)-ATPase is co-expressed with a mutant of this type, it becomes arrested in the same membranes and growth stops; thus, the mutation acts genetically in a dominant lethal fashion (10–13).

Recent experiments have suggested that the exact point of arrest in the ER may vary with the severity of the \( \text{pma1} \) mutation. Least seriously affected among those studied to date is \( \text{G381A} \), only three residues downstream from the critical Asp
that is phosphorylated by ATP during the catalytic cycle. The G381A polypeptide displays an intermediate folding defect and is transiently arrested in the ER; it then escapes via the secretory vesicles to the plasma membrane, from which it is recycled into the nature of the ER quality control process has come from screening an insertion genomic library for suppressors of the dominant lethal behavior of D378N (17). This approach has yielded a gene called EPS1 (ER-retained \textit{pma1} suppressing) that, when disrupted, prevents degradation of the D378N polypeptide and allows it to reach the cell surface. The product of the EPS1 gene belongs to the protein disulfide isomerase family and may act as a membrane-bound chaperone. Its specificity for misfolded H\textsuperscript{+}-ATPase is not fully understood, but deleting EPS1 has little or no effect on the biogenesis of the wild-type H\textsuperscript{+}-ATPase or on the retention of other proteins that normally reside in the ER.

\section*{Lipid Rafts Help to Carry H\textsuperscript{+}-ATPase through Golgi to Plasma Membrane}

Lipid rafts, consisting of tightly packed sphingolipids and cholesterol, were first observed in mammalian cells, where they form in the Golgi and are believed to play a role in membrane trafficking and cell signaling (reviewed in Ref. 30). Recently, Bagnat et al. (31) have isolated lipid rafts from yeast cells by flotation as detergent-insoluble glycolipid-enriched complexes. The rafts, which appear as early as the ER in yeast, resemble the plasma membrane in having a high content of sphingolipids, ergosterol, and saturated phospholipids; and mass spectrometry has shown them to contain Pma1 ATPase and at least one other protein (Gas1p, a glycosphospholipid-anchored protein of unknown function) bound for the cell surface. Conspicuous sensitivity to trypsin, this ATPase is very poorly folded and becomes arrested in invasive “sausage-like” structures, which are derived from the ER (10, 13) and may represent proliferation of the vesicular-tubular elements. Unlike G381A, D378N fails to escape to the Golgi and is translocated back into the cytoplasm for degradation by the proteasome (17). Insight into the nature of the ER quality control process has come from screening an insertion genomic library for suppressors of the dominant lethal behavior of D378N (17). This approach has yielded a gene called EPS1 (ER-retained \textit{pma1} suppressing) that, when disrupted, prevents degradation of the D378N polypeptide and allows it to reach the cell surface. The product of the EPS1 gene belongs to the protein disulfide isomerase family and may act as a membrane-bound chaperone. Its specificity for misfolded H\textsuperscript{+}-ATPase is not fully understood, but deleting EPS1 has little or no effect on the biogenesis of the wild-type H\textsuperscript{+}-ATPase or on the retention of other proteins that normally reside in the ER.

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Topography of the yeast plasma membrane H\textsuperscript{+}-ATPase, based on two- and three-dimensional crystallography of related ATPases at resolutions of 8 Å (2–4) and 2.6 Å (5). N and C termini are located in the cytoplasm. Zig-zag lines represent regions predicted to have \(\alpha\)-helical secondary structure; red circles mark positions at which mutations have been found to disrupt protein folding, block biogenesis, and/or lead to a dominant lethal phenotype (see text).}
\end{figure}

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Genes whose products are implicated in biogenesis and degradation of the Pma1 ATPase. PM, plasma membrane; SV, secretory vesicles.}
\end{figure}
Protection from trypsinolysis is afforded by ligands such as MgADP, and ATPase is catalytically active in the ER, although it can be phosphatase, as well as secreted exoglucanase (Exg1p) (35). Wall form of endoglucanase (Bgl2p); the minor vesicle population, together with the cell membrane. In fact, yeast contains at least two subpopulations of secretory vesicles, similar in size (100 nm) but separable by density gradient centrifugation (16). Eight of the sop suppressors match known VPS (vacuolar protein sorting) genes that control the biogenesis of newly synthesized vacuolar proteins; others are not absolutely required for vacuolar biogenesis but still have noticeable effects on Golgi-to-endosome or endosome-to-vacuole protein trafficking (16, 18). Based on these results, which point to a central cytoplasmic loop and G789S at the extracytoplasmic end of transmembrane segment 8) and displays a temperature-sensitive defect in H\(^+\)-ATPase biogenesis (14). At 25 °C, Pma1–7p is able to reach the plasma membrane and support growth, whereas at 37 °C, it is degraded rapidly in the vacuole. Two different approaches have been used to isolate suppressors of pma1–7 with the aim of uncovering novel components of the vacuolar degradation pathway. In the first, screening with a high-copy genomic library yielded a pair of related genes (AST1 and AST2, for ATPase stabilizing) whose products, when overexpressed, cause Pma1–7p to bypass the degradation pathway and travel to the plasma membrane (14). Further examination showed Ast1p to be a peripheral membrane protein, which co-fractionates with detergent-insoluble material that may correspond to the recently identified lipid rafts (see above).

In a subsequent study, screening with an insertional library yielded 16 different sop (suppressors of pma1–7) genes, which, when disrupted, could re-route Pma1–7p to the plasma membrane (16). Eight of the sop suppressors match known VPS (vacuolar protein sorting) genes that control the biogenesis of newly synthesized vacuolar proteins; others are not absolutely required for vacuolar biogenesis but still have noticeable effects on Golgi-to-endosome or endosome-to-vacuole protein trafficking (16, 18). Based on these results, which point to a central and complex role of the endosomal system in dictating the fate of the mutant Pma1–7 H\(^+\)-ATPase, the authors have proposed the existence of an alternative endosome-to-surface pathway (16, 18) (Fig. 2).

**Mutant H\(^+\)-ATPases That Escape ER Undergo a Second Quality Control Step in Golgi**

Recent studies by Chang and co-workers have pointed to a Golgi-based quality control process, which recognizes abnormal H\(^+\)-ATPases that have avoided proteasomal degradation and delivers them to the vacuole for proteolysis. One such mutant, pma1–7, carries two amino acid substitutions (P434A in the central cytoplasmic loop and G789S at the extracytoplasmic end of transmembrane segment 8) and displays a temperature-sensitive defect in H\(^+\)-ATPase biogenesis (14). At 25 °C, Pma1–7p is able to reach the plasma membrane and support growth, whereas at 37 °C, it is degraded rapidly in the vacuole. Two different approaches have been used to isolate suppressors of pma1–7 with the aim of uncovering novel components of the vacuolar degradation pathway. In the first, screening with a high-copy genomic library yielded a pair of related genes (AST1 and AST2, for ATPase stabilizing) whose products, when overexpressed, cause Pma1–7p to bypass the degradation pathway and travel to the plasma membrane (14). Further examination showed Ast1p to be a peripheral membrane protein, which co-fractionates with detergent-insoluble material that may correspond to the recently identified lipid rafts (see above).

In a subsequent study, screening with an insertional library yielded 16 different sop (suppressors of pma1–7) genes, which, when disrupted, could re-route Pma1–7p to the plasma membrane (16). Eight of the sop suppressors match known VPS (vacuolar protein sorting) genes that control the biogenesis of newly synthesized vacuolar proteins; others are not absolutely required for vacuolar biogenesis but still have noticeable effects on Golgi-to-endosome or endosome-to-vacuole protein trafficking (16, 18). Based on these results, which point to a central and complex role of the endosomal system in dictating the fate of the mutant Pma1–7 H\(^+\)-ATPase, the authors have proposed the existence of an alternative endosome-to-surface pathway (16, 18) (Fig. 2).

**By the Time H\(^+\)-ATPase Reaches Secretory Vesicles, It Is Capable of ATP-dependent Proton Translocation**

Like most other cell surface or secreted proteins, wild-type H\(^-\)-ATPase travels from the Golgi to the cell surface via secretory vesicles, which bud from the Golgi to fuse with the plasma membrane. In fact, yeast contains at least two subpopulations of secretory vesicles, similar in size (100 nm) but separable by equilibrium sedimentation (35). The ATPase is found in the major vesicle population, together with the cell wall form of endoglucanase (Bgl2p); the minor vesicle population contains periplasmic enzymes such as inverase and acid phosphatase, as well as secreted exoglucanase (Exg1p) (35). As described above, there is no clear evidence that the H\(^+\)-ATPase is catalytically active in the ER, although it can be protected from trypsinolysis there by ligands such as MgADP and orthovanadate. In the secretory vesicles, however, the ATPase is clearly able to hydrolyze ATP and pump protons at rates comparable with those seen in the plasma membrane; this property allows isolated secretory vesicles to be used as a convenient expression system for site-directed pma1 mutants (36).

**Abnormal H\(^+\)-ATPases That Reach Plasma Membrane Are Retrieved by Endocytosis and Sent to Vacuole for Degradation**

Wild-type Pma1 H\(^+\)-ATPase turns over with a half-life of 11 h, making it one of the most stable constituents of the yeast plasma membrane (37). By contrast, the G381A mutant polypeptide has a half-life of only 1 h, leaving the plasma membrane by way of the endocytic pathway to undergo degradation in the vacuole. This finding suggests the existence of a third quality control mechanism at the yeast cell surface. The precise mechanism for recognizing misfolded H\(^+\)-ATPase is unknown, but a protein known variously as End4p/Sla2p/ Mop2p has been shown to be required for efficient endocytosis of G381A.2 End4p/Sla2p/Mop2p is a component of the actin cytoskeleton and may help in the formation of endocytic vesicles by stimulating actin depolymerization at the site of vesicle budding (Fig. 2) (reviewed in Ref. 38). Interestingly, mutations in the END4/SLA2/MOP2 gene have been selected by their ability to lower the abundance of wild-type ATPase at the cell surface (15). There is considerable evidence that ubiquitination plays an essential role in the endocytosis of short-lived plasma membrane proteins (reviewed in Refs. 39 and 40) but whether it is similarly involved in the endocytosis of mutant or wild-type H\(^+\)-ATPase remains to be established.

**Summary and Prospects for Future Work**

Taken together, the research described above has begun to define the path by which a highly abundant plasma membrane protein, the yeast H\(^-\)-ATPase, travels from the ER to the cell surface. Further work should clarify the relationship between the sorting of newly synthesized ATPase into lipid rafts and the packaging of the ATPase into the appropriate subset of COPII vesicles. The location of these events within the ER will also be of interest, as well as the functional role of the exaggerated vesicular-tubular elements that form in pma1 mutants such as G381A. In parallel, further research is needed to understand the significance of the stepwise phosphorylation events that accompany movement of Pma1 ATPase along the secretory pathway. Finally, Pma1p can serve as a valuable model for understanding quality control during biogenesis because abnormal forms are recognized and removed at three successive points along the pathway. These and other aspects of ATPase biogenesis promise to be active subjects for study in the years to come. For further information on members of the P-type ATPase family, the reader is directed to the accompanying review of Na\(^+\),K\(^+\)- and H\(^+\),K\(^+\)-ATPases by Dunbar and Caplan (41).

Acknowledgment—We thank Dr. Michael Caplan for critical reading of this manuscript.

REFERENCES


structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution. *Nature* 405, 647–655
The Yeast Pma1 Proton Pump: a Model for Understanding the Biogenesis of Plasma Membrane Proteins
Thierry Ferreira, A. Brett Mason and Carolyn W. Slayman

doi: 10.1074/jbc.R100022200 originally published online June 12, 2001

Access the most updated version of this article at doi: 10.1074/jbc.R100022200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 41 references, 23 of which can be accessed free at http://www.jbc.org/content/276/32/29613.full.html#ref-list-1