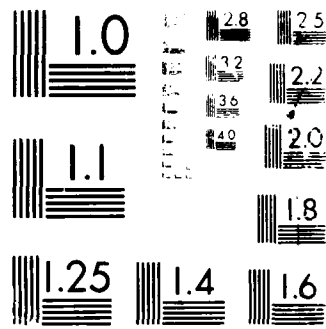


AD-A194 782 MEMBRANE VOLTAGE EFFECTS ON PROTON TRANSPORT BY A YEAST 1/1
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OF NEW YORK INC DEPT OF BIOCHEMISTRY D S PERLIN
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<p>A structural mechanism for proton transport is being pursued by examining the effects of membrane voltage on proton translocation by normal and mutant H⁺-ATPases from yeast plasma membranes. As a first step, a large collection of mutants with single-site mutations in the H⁺-ATPase gene (<u>PMA1</u>) was isolated from mutagenized yeast by selecting for resistance to hygromycin B. The <u>pmal</u> mutants were sensitive to low pH, weak acid loading, osmotic pressure and NH₄⁺; these phenotypes were complemented by expression of plasmid-associated normal <u>PMA1</u>. The mutant H⁺-ATPases were found to have kinetic defects that decreased K_m and V_{max} and altered sensitivity to mechanism-specific inhibitors like vanadate. The mutant enzymes still transported protons but failed to maintain a normal hyperpolarized level of the membrane potential. In the next year, we plan to study the voltage dependence of proton transport and ATP hydrolysis by normal and <u>pmal</u> mutant H⁺-ATPases.</p>			
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INTRODUCTION

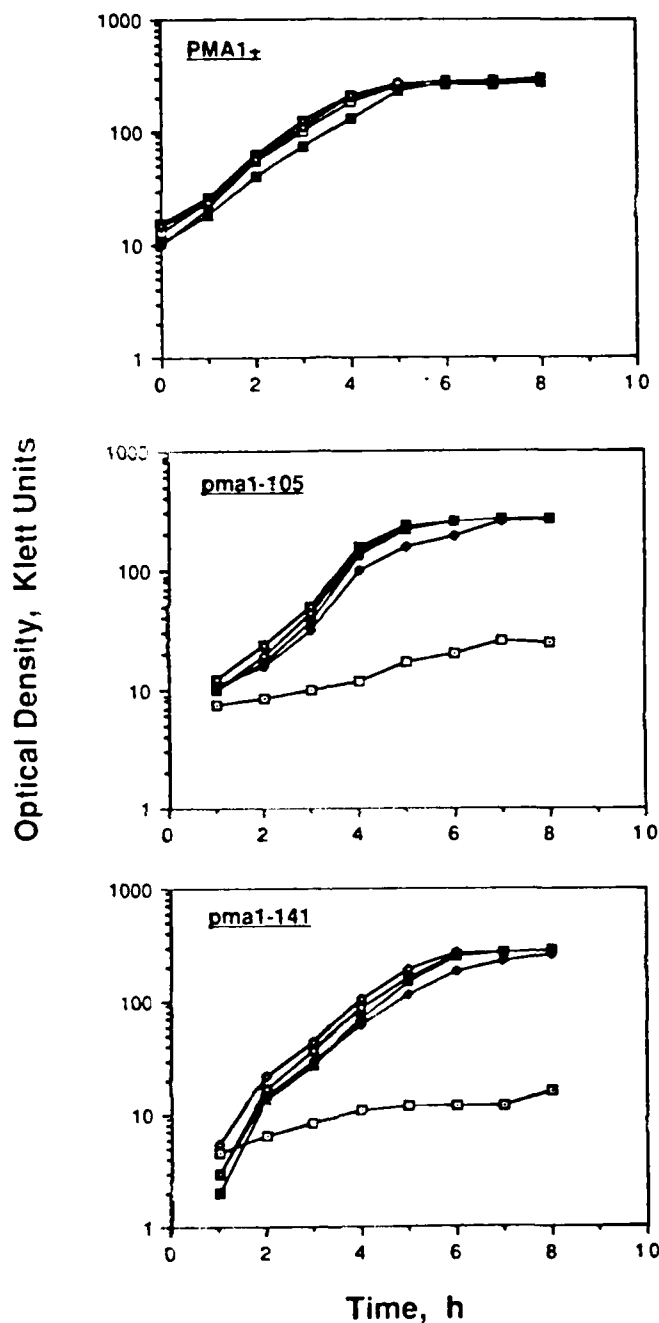
The objective of this project is to study membrane voltage effects on electrogenic proton transport by normal and mutant forms of the H⁺-ATPase from yeast plasma membranes. This information, coupled with mutant mapping studies, will be used to describe a structurally-distinct proton translocation pathway.

The initial goal of this project was to isolate a collection of H⁺-ATPase-defective mutants. The yeast H⁺-ATPase is an electrogenic proton pump that plays a vital role in nutrient uptake and intracellular pH regulation. The gene encoding this enzyme, PMA1, was found to be essential for growth. (Serrano, R., Kielland-Brandt, M.C. and Fink, G.R. 1986 Nature 319, 689-693). The cellular importance of the H⁺-ATPase mandates that viable pma1 mutants can only arise from mutations resulting in partially active or conditionally inactive enzymes; past attempts to isolate pma1 mutants were often thwarted by the lack of a suitable selection routine. Recently, in collaboration with Dr. James Haber of Brandeis University, we described a positive selection procedure for isolating pma1 mutants from S. cerevisiae based on resistance of UV-treated cells to the aminoglycoside antibiotic hygromycin B. (McCusker, J.E., Perlin, D.S. and Haber, J.E. 1987 Mol. Cell. Biol. 7, 4082-4088). Our working hypothesis was that uptake of this antibiotic was linked to the electrochemical proton gradient and resistant mutants would show defects in the pH gradient and/or the membrane potential. Our first year was spent characterizing the more than 75 mutants isolated and identifying potential transport-defective mutants.

PROGRESS REPORT

1. Properties of pma1 mutants. Most pma1 mutants were unable to tolerate acid loading conditions which included growth at low external pH (Fig. 1) or growth in the presence of large amounts of weak acids. The mutants were also very sensitive to NH₄⁺ and medium osmotic pressure; these phenotypes were all complemented by plasmid-associated normal PMA1. Intragenic complementation of pma1 mutants suggested that the H⁺-ATPase is, at the very least, a dimeric enzyme.

2. Biochemical properties of pma1 mutants. Expression and assembly of the H⁺-ATPase appeared normal in the majority of pma1 mutants since wild type levels of intact enzyme, M_r=100,000, were



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Fig. 1. Effect of medium pH on growth of pma1 mutants. Cell growth of wildtype (PMA1⁺), pma1-105 and pma1-141 was monitored in buffered growth medium adjusted to pH 7.5 (◆), pH 6.5 (■), pH 5.5 (◼), pH 4.5 (◆) and pH 3.5 (◻).

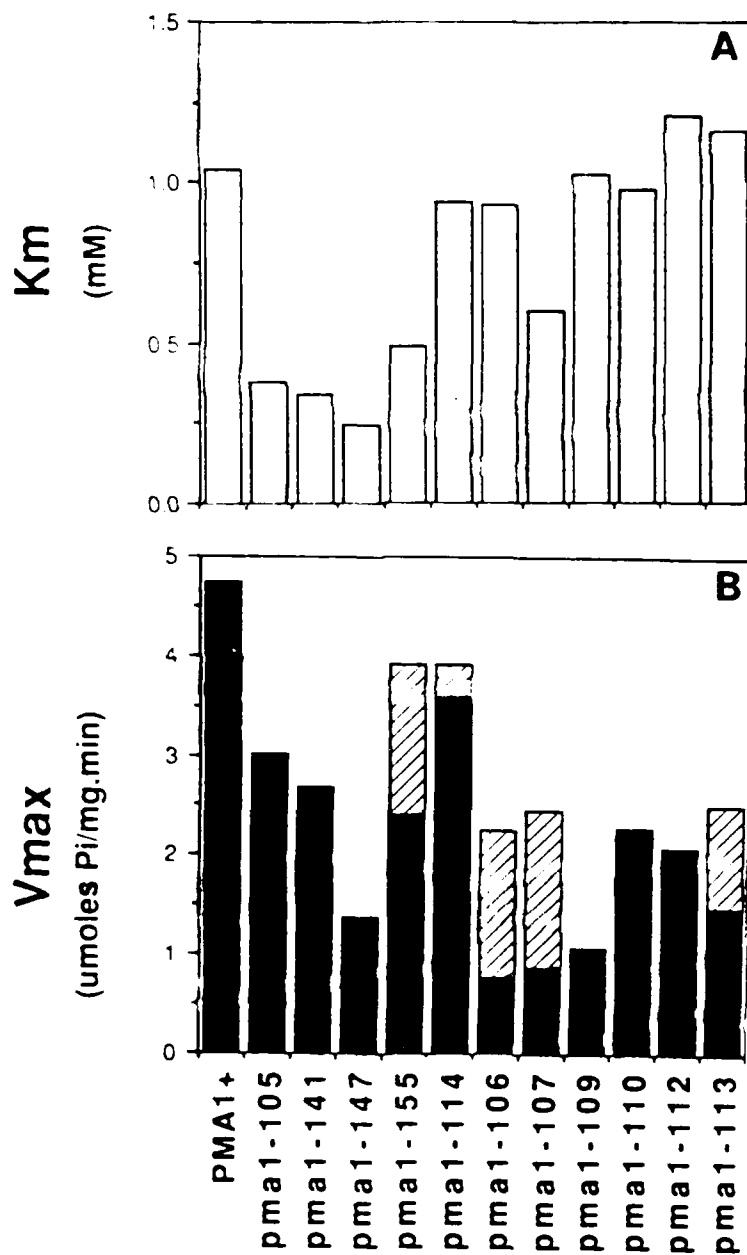


Fig. 2. Kinetic properties of mutant enzymes. Kinetic parameters Km (panel A) and Vmax (panel B) were determined for mutant enzymes at pH 6.5. The cross-hatch area in panel B represents Vmax values normalized to control levels of intact enzyme.

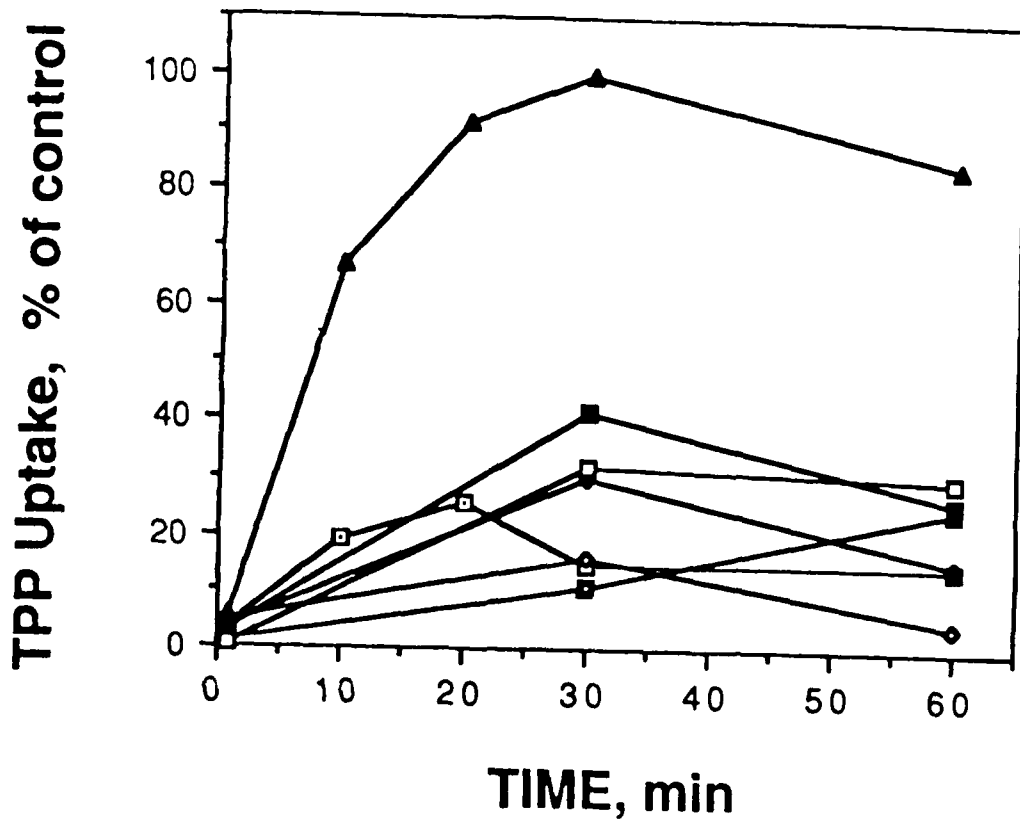


Fig. 3. Uptake of [^{14}C]-TPP by *pma1* mutants. Steady-state uptake of [^{14}C]-TPP by wild type (▲) and *pma1*-101 (◆), *pma1*-105 (◻), *pma1*-114 (◼), *pma1*-141 (◼), *pma1*-147 (◻) and *pma1*-155 (◊) mutants in the presence of glucose was determined by a rapid filtration assay. Uptake from de-energized cells was subtracted from these plots.

found. Three types of kinetic defects resulting in a decreased K_m and/or V_{max} were found (Fig. 2); enzymes from two strains, pma1-105 and -141 which were growth inhibited by low pH, showed a precipitous decline in V_{max} below pH 6.5. The H^+ -ATPase is strongly inhibited by vanadate and three mutant enzymes, pma1-105, -141 and -147 were found to be vanadate-insensitive. Intragenic second-site suppression of these primary mutations led to the isolation of partial revertants with restored vanadate sensitivity. Vanadate-insensitive enzymes formed normal phosphorylated intermediates but appeared to show differences in steady-state levels of E_1 and E_2 conformational intermediates during catalysis.

3. Transport behavior of pma1 mutants. It was found that net proton efflux, as measured by whole cell medium acidification in the presence of 25 mM KCl, was nearly identical for wild type and pma1 mutant cells. However, in the absence of added KCl, the initial rate and final extent of net proton efflux for wild type was considerably less than that of the pma1 mutants. Changes in proton leak pathways were not considered likely since passive proton conductance and intracellular buffering capacity were unaltered in the mutants. The cellular membrane potential was identified as an essential factor in regulating proton fluxes and was found from [^{14}C]-tetraphenylphosphonium distribution studies to be strongly depolarized in pma1 mutants (Fig. 3). Depolarization of the membrane potential also helped explain resistance of pma1 mutants to yeast killer toxin. The action of yeast killer toxin has been linked to a hyperpolarized membrane state.

4. Perspectives and future goals. Our finding that hygromycin B-resistant pma1 mutants show defects in the cellular membrane potential suggests that hygromycin B is an effective selective agent for isolating depolarized cells. Changes in cellular membrane potential are a direct consequence of mutations within PMA1 that alter the H^+ -ATPase. However, it needs to be established whether the H^+ -ATPase alone is responsible for the observed depolarization or whether non-ATPase-mediated electrical leak pathways also contribute. One exciting possibility is that pma1 mutant enzymes have altered charge-transfer properties. To examine this possibility, normal and mutant H^+ -ATPases are being purified and reconstituted in liposomes, and the effects of applied membrane voltage are being used to probe differences in proton transport and ATP hydrolysis.

PUBLICATIONS

1. McCusker, J.H., Perlin, D.S. and Haber, J.E. 1987 Pleiotropic plasma membrane ATPase mutations of Saccharomyces cerevisiae. Mol. Cell. Biol. 7, 4082-4088
2. Perlin, D.S., McCusker, J.H. and Haber, J.E. 1988 Defective H⁺-ATPase of hygromycin B-resistant pma1 mutants from Saccharomyces cerevisiae. J. Biol. Chem., submitted
3. Perlin, D.S., Brown, C.L. and Haber, J.E. 1988 Membrane potential defect in hygromycin B-resistant pma1 mutants of Saccharomyces cerevisiae. J. Biol. Chem., submitted.

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