Yeast as a Model System for Cystinosis: Alterations in V-ATPase function in *ers1-Δ*

Progress Report
November 4, 2009
Seasson P. Vitiello and David A. Pearce

The single-celled eukaryote *Saccharomyces cerevisiae,* or budding yeast, is a useful model system because it is amenable to genetic manipulations and biochemical analyses. The functional ortholog of cystinosin is Ers1p, which is encoded by the *ERS1* gene. We aimed to identify and study the cellular defects that occur when *ERS1* is deleted in yeast. In our January 2009 progress report, we described a forward genetic screen that used an *ERS1* deletion strain, *ers1-Δ.* This screen revealed cold resistant phenotypes that suggested alterations in vacuolar pH and the vacuolar H^+-ATPase (V-ATPase). The V-ATPase is a complex of proteins consisting of a membrane bound V_0 subunit and a soluble, cytosolic V_1 subunit. Association of the V_1 and V_0 subunits results in ATP hydrolysis-driven proton transport, which establishes the acidity and proton-motive force required for transport of various substrates, including cystine. As previously reported, although vacuolar pH is more acidic, there is a 20% decrease in V-ATPase subunit association in *ers1-Δ* as compared to $ERS1^+$ cells. The association was measured by co-immunoprecipitation of the two subunits, followed by Western blotting of the eluate using antibodies specific for components of the V_1 and V_0 subunits. Densitometry was performed on the Western blot to calculate percent assembly. Subunit dissociation and re-association after dissociation was measured in a similar manner. We found that subunit dissociation is unchanged and curiously, subunit re-association following dissociation is also unchanged (Figure 1). The decrease in subunit association is not due to decreased V_1 or V_0 subunit protein levels in the cells or at the vacuole (Figure 2). Two-dimensional blue native gel electrophoresis, a method to separate complexes in enriched organelle fractions, also showed no difference in protein levels in enriched vacuolar fractions (data not shown). We aim to characterize why association, but not re-association, is different in *ers1-Δ* as compared to $ERS1^+$.

We speculate that the V-ATPase is being down-regulated through decreased subunit association to correct for the decreased vacuolar pH, and this decreased subunit association results in decreased V-ATPase-dependent H^+ pumping and ATP hydrolysis in *ers1-Δ*. Future experiments will measure these activities in *ers1-Δ*. An *ers1-Δcup5-Δ* double deletion strain was phenotypically similar to the *cup5-Δ* single deletion strain, which supports this hypothesis (data not shown), although more experiments need to be performed to verify this. Further analysis of the *ers1-Δcup5-Δ* double deletion strain may give clues into the relationship between *ERS1* and the V-ATPase. Experiments will include measuring vacuolar pH and V-ATPase activity. In addition, two plasmids were recently constructed from the yeast shuttle vector pRS316, a single copy, centromeric plasmid with *URA3* auxotrophic selection. The first construct contains 520 base-pairs upstream of *ERS1,* followed by the *ERS1* open reading frame. The second construct contains 520 base-pairs upstream of *ERS1,* followed by *CTNS* cDNA. These plasmids will be used to complement the above phenotypes. Alterations in the V-ATPase can affect many

cellular processes, including autophagy. Macroautophagy, which is dependent on V-ATPase function, does not appear to be compromised in *ers1-Δ* as tested by a colony formation assay (data not shown). Cells were incubated in low nitrogen containing media and plated to determine cell viability in those conditions. Carboxypeptidase Y (CPY) trafficking can also be affected by alterations at the vacuole, specifically protein trafficking, where CPY is either incorporated into the vacuole or, if there is a problem with the vacuole, it is secreted into the extracellular area. The amount of CPY that is secreted can be measured by growing cells on a nitrocellulose membrane, washing the cells away, and Western blotting the membrane using an antibody that is specific to CPY. We saw no CPY secretion defect in *ers1-Δ,* indicating that this particular pathway is not affected by loss of *ERS1* (data not shown). Other pathways that may be affected by V-ATPase dysfunction in *ers1-Δ* are degradation of macromolecules within the vacuole, transport of small molecules across the vacuolar membrane, vesicular trafficking and endocytosis, and mitochondrial function. Our long term goal is to analyze these pathways in *ers1-Δ,* eventually studying these alterations in higher eukaryotic model systems.

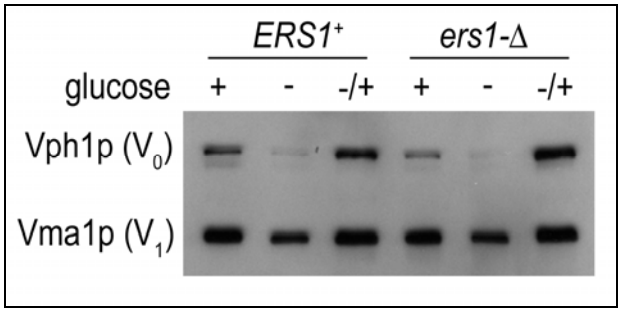

Figure 1. V-ATPase association is decreased, but dissociation and re-association is normal in *ers1-Δ*. Association of the V_1 and V_0 subunits was measured by co-immunoprecipitation of the subunits from cell lysates. The V_1 subunit was immunoprecipitated and the eluate was subjected to SDS-PAGE followed by Western blotting for the V_0 and V_1 subunit constituents Vph1p and Vma1p, respectively. The co-immunoprecipitation was done in the presence of glucose (+) when the V-ATPase is fully assembled in the *ERS1*$^+$ strain, in the absence of glucose (-) when most of the subunits are dissociated, and in the absence of glucose followed by addition of glucose (-/+) to monitor re-association of the complex.

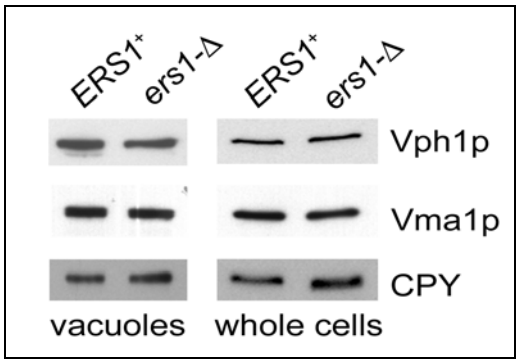

Figure 2. V-ATPase subunit protein levels are unchanged in *ers1-Δ* as compared to *ERS1*$^+$. V-ATPase subunit levels were measured in cell lysate and enriched vacuole preparations by SDS-PAGE and subsequent Western blotting for Vma1p and Vph1p, constituents of the V_1 and V_0 subunits, respectively. Carboxypeptidase Y (CPY), a soluble vacuolar protein that is not associated with V-ATPase function, was used as a control.

This data combined with our previous reports on our studies are currently being assembled into a manuscript for submission. We hope to pass this manuscript on to you once it has been accepted for publication.