The long physiological reach of the yeast vacuolar H\(^+\)-ATPase

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Abstract V-ATPases are structurally conserved and functionally versatile proton pumps found in all eukaryotes. The yeast V-ATPase has emerged as a major model system, in part because yeast mutants lacking V-ATPase subunits (vma mutants) are viable and exhibit a distinctive Vma- phenotype. Yeast vma mutants are present in ordered collections of all non-essential yeast deletion mutants, and a number of additional phenotypes of these mutants have emerged in recent years from genomic screens. This review summarizes the many phenotypes that have been associated with vma mutants through genomic screening. The results suggest that V-ATPase activity is important for an unexpectedly wide range of cellular processes. For example, vma mutants are hypersensitive to multiple forms of oxidative stress, suggesting an antioxidant role for the V-ATPase. Consistent with such a role, vma mutants display oxidative protein damage and elevated levels of reactive oxygen species, even in the absence of an exogenous oxidant. This endogenous oxidative stress does not originate at the electron transport chain, and may be extra-mitochondrial, perhaps linked to defective metal ion homeostasis in the absence of a functional V-ATPase. Taken together, genomic data indicate that the physiological reach of the V-ATPase is much longer than anticipated. Further biochemical and genetic dissection is necessary to distinguish those physiological effects arising directly from the enzyme’s core functions in proton pumping and organelle acidification from those that reflect broader requirements for cellular pH homeostasis or alternative functions of V-ATPase subunits.

Keywords V-ATPase · Yeast · vma mutant · Acidification · Genomic · Oxidative stress

Introduction

Vacuolar proton-translocating ATPases (V-ATPases) are functionally diverse proton pumps with a highly conserved structure (Kane 2006; Nishi and Forgac 2002). In all eukaryotic cells, V-ATPases are responsible for acidification of a variety of organelles, including lysosomes/vacuoles, endosomes, and the late Golgi apparatus. In certain cells, they have also been adapted to export protons from the cytosol across the plasma membrane (Breton and Brown 2007; Wieczorek et al. 1999). V-ATPases contribute to cellular pH control in all of these different cellular contexts.

All eukaryotic V-ATPases have a very similar structure, consisting of approximately 14 subunits arranged into two subcomplexes: a peripheral membrane subcomplex called V\(_1\) and an integral membrane subcomplex designated V\(_0\) (Nishi and Forgac 2002). In mammalian cells, many of these subunits exist as several tissue- or organelle-specific isoforms, but in yeast, all subunits except the V\(_0\) “a” subunit are encoded by a single gene (Fig. 1). Deletion of any one of these genes, any of four assembly factors dedicated to the V-ATPase (Davis-Kaplan et al. 2006; Graham et al. 1998), or both of the a subunit isoforms, results in a very similar phenotype. This Vma- phenotype, is characterized by a distinct pattern of pH and calcium sensitive growth, metal ion sensitivity, and inability to grow on non-fermentable carbon sources (Kane 2006). In contrast, complete loss of V-ATPase activity in eukaryotes other than fungi is lethal, often at very early stages of development (Allan et al. 2005; Sun-Wada et al. 2000). Null
mutations of subunit isoforms permit viability in some cases in metazoans, and result in specific defects characteristic of the sphere of influence of V-ATPases containing that specific isoform (Borthwick and Karet 2002).

Because yeast vma deletion mutants are viable, it is possible to assess the downstream consequences of abrogating V-ATPase function from the properties of the vma mutants. Both direct analysis of the vma mutants and phenotypic screens of ordered yeast deletion mutant arrays have revealed that loss of V-ATPase activity has unexpectedly diverse consequences. These results indicate that tight-control of pH and/or “moonlighting” functions of V-ATPase subunits are intertwined with a large number of cellular processes. In this review, we will describe the diverse functional connections to the V-ATPase revealed by yeast genomic screens in recent years, and then focus on recent data suggesting that V-ATPases may help to provide resistance to oxidative stress.

Genomic screens highlight widespread defects in vma mutants

The development of ordered deletion mutant arrays lacking individual, marked, non-essential yeast genes has permitted many non-biased genomic screens for specific growth phenotypes and inhibitor sensitivities (Giaever et al. 2002). In these screens, the collection of almost 5,000 non-essential deletion mutants is tested for growth under varied conditions, and mutants with selectively compromised growth are identified. The resulting collection of mutants is further analyzed to look for enrichment of certain classes of genes. Statistically enriched classes can identify a complex or pathway that enables wild-type cells to grow under the conditions tested. Although it is certainly true that mutations can rather indirectly impact the ability of cells to grow under any given set of conditions, it is a significant advantage that genomic screens can potentially highlight the full scope of gene products required for growth, and provide evidence of important functional connections that were not appreciated previously. For example, this approach was used to screen the haploid non-essential deletion collection for all mutants exhibiting the classical Vma− phenotype, specifically, compromised growth at high pH and/or high calcium concentrations (Sambade et al. 2005; Serrano et al. 2004). These genomic screens identified almost all of the previously characterized V-ATPase subunits (in our screen, the strains lacking the other subunits were not represented in our library), as well as a previously unidentified subunit of the enzyme (Sambade and Kane 2004) and a number of potential regulators (Sambade et al. 2005). A parallel screen also identified a novel assembly factor for the V-ATPase (Davis-Kaplan et al. 2004). All of these screens identified other strains that may have compromised vacuolar acidification.

In addition to their expected prominence in the total set of deletion mutants sensitive to elevated pH and high calcium, vma mutants emerged as a major class of mutants that display metabolic and morphological aberrations and sensitivity to a number of other treatments. As summarized in Table 1, vma mutants were over-represented in genomic screens for: (1) sensitivity to multiple drugs, (2) sensitivity to elevated metal ion concentration, (3) sensitivity to limited iron availability, (4) sensitivity to both low and high extracellular calcium, (5) sensitivity to alcohol stress, (6) poor growth on high salt, (7) aberrant vacuolar morphology, (8) excess glycogen accumulation, (9) sensitivity to DNA damaging reagents, and (10) sensitivity to multiple forms of oxidative stress (see Table 1 for references to specific screens). Some of these results may be readily explained by established roles of the vacuole in metal ion and calcium homeostasis, in nutrient storage and in sequestration of multiple metabolites and toxins (Klionsky et al. 1990). Additionally, V-ATPases that reside outside the vacuole may also have critical functions, as suggested by a recent study comparing the phenotypic consequences of V-ATPase activity loss to effects of losing Pmr1p, a calcium pump localized in the Golgi (Yadav et al. 2007). Other results, however, such as multi-drug sensitivity and sensitivity to DNA damaging agents, are harder to incorporate into our current understanding of V-ATPase function, and suggest that the influence of the V-ATPase in overall cell physiology is far more extensive than anticipated.