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Minireview

# Regulation of V-ATPases by reversible disassembly

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**Abstract V-ATPases consist of a complex of peripheral subunits containing catalytic sites for ATP hydrolysis, the $V_1$ sector, attached to several membrane subunits containing a proton pore, the $V_0$ sector. ATP-driven proton transport requires structural and functional coupling of the two sectors, but in vivo, the interaction between the $V_1$ and $V_0$ sectors is dynamic and is regulated by extracellular conditions. Dynamic instability appears to be a general characteristic of V-ATPases and, in yeast cells, the assembly state of V-ATPases is governed by glucose availability. The structural and functional implications of reversible disassembly of V-ATPases are discussed.**



*Key words:* V-ATPase; Proton pump; Enzyme regulation; Acidification; Vacuole; *Saccharomyces cerevisiae*

## 1. Introduction

Vacuolar proton-translocating ATPases (V-ATPases) are highly conserved proton pumps found on intracellular membranes of all eukaryotic cells and at the plasma membrane of a subset of cells [1–3]. V-ATPases couple hydrolysis of cytosolic ATP to proton transport out of the cytosol, resulting in acidification of intracellular compartments such as the vacuole/lysosome, the endosome and the Golgi apparatus and energization of the plasma membrane in a variety of cells. The central role of V-ATPases in cell physiology is only beginning to be appreciated. V-ATPases have adapted to a remarkably wide variety of physiological roles ranging from controlling cytosolic pH homeostasis to driving secondary transport of ions and nutrients to regulating protein sorting [1–3].

V-ATPases consist of a complex of peripheral subunits, known as the $V_1$ sector, attached at the cytoplasmic face of membranes to a complex of integral membrane and tightly associated peripheral proteins known as the $V_0$ sector [1,4–7]. In yeast cells, the $V_1$ sector is comprised of eight polypeptides and the $V_0$ sector contains five; other V-ATPases appear to have very similar subunit compositions [6,8]. A structural model of the yeast enzyme is shown in Fig. 1. The 'catalytic core' of the enzyme includes the catalytic subunits (subunit A) responsible for ATP hydrolysis, closely associated regulatory subunits (subunit B) that may both contribute residues to the active site and bind nucleotides on their own and proteolipid subunits (subunits c, c′ and c″) that associate to form the proton pore [1,4–8]. The catalytic and regulatory subunits are part of the $V_1$ sector and the proteolipid subunits are in the $V_0$ sector. These subunits show significant sequence homology with the catalytic, regulatory and proteolipid subunits of F-type ATPases [9], except that there appear to be three different but homologous proteolipid subunits present in each V-ATPase complex while the proton pore of F-ATPases is comprised of a single type of proteolipid subunit [10]. The fundamentally similar catalytic mechanisms in the F- and V-type ATPases reflect the sequence homology between subunits of the catalytic core [1,4].

The remaining subunits of F- and V-type ATPases form all or part of two to three stalks and are involved, either directly or indirectly, with structural and functional coupling of the peripheral $F_1$ or $V_1$ and membrane $F_0$ or $V_0$ sectors [7,11–15]. These subunits are conserved within F- or V-type ATPases, but show much less similarity between F- and V-type ATPases, suggesting some fundamental differences between the two classes of enzymes [1,7]. One fundamental difference is the stability of the $F_1F_0$ and $V_1V_0$ complexes in the cell. The $F_1$ and $F_0$ sectors associate to form a stable complex in the cell and are rarely, if ever, present in their dissociated form. In contrast, dissociated $V_1$ and $V_0$ sectors are present in dynamic equilibrium with membrane-bound $V_1V_0$ complexes and this equilibrium can be perturbed as a major mechanism of regulation [6,16].

## 2. Reversible disassembly of V-ATPases

Although free $V_1$ and $V_0$ sectors had been found in a number of different eukaryotic cells [17–20], their relationship with fully assembled V-ATPases was unclear for a number of years. The structural independence of the $V_1$ and $V_0$ sectors was clear from a number of different types of experiments. Dissociation of the enzyme into free $V_1$ and $V_0$ sectors could be achieved with relatively mild conditions in vitro, but dissociated $V_1$ lost ATP hydrolytic activity and the free $V_0$ did not appear to be an open proton pore [18,21]. Mutant yeast cells lacking one of the V-ATPase subunits were able to assemble either an intact $V_1$ or $V_0$ sector in vivo, even in the absence of the other sector [19,20]. The free $V_1$ and $V_0$ sectors present in wild-type cells in vivo were generally believed to be either assembly intermediates or biochemical artifacts arising from disruption of complexes during cell lysis. This picture changed in 1995, when experiments done independently in yeast and in insect cells revealed that fully assembled V-ATPase complexes could be induced to reversibly dissociate into free $V_1$ and $V_0$ complexes in vivo by changes in extracellular conditions [22,23].

In yeast cells, disassembly of the V-ATPase was induced by brief glucose deprivation [22]. In cells grown in a rich carbon

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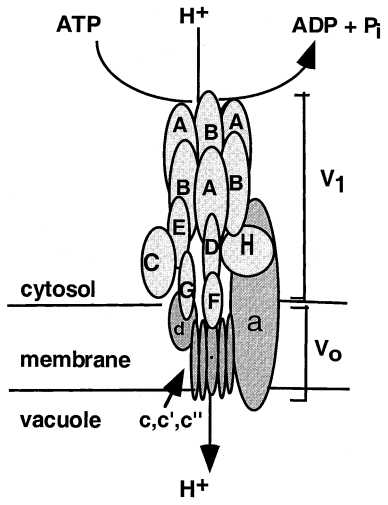


Fig. 1. Structural model of the yeast V-ATPase. $V_1$ subunits are shown in light gray and $V_0$ subunits are shown in dark gray. Letter designations for subunits are those proposed in [1].

source such as glucose, 60–70% of the $V_0$ complexes were assembled with $V_1$ subunits, but within 5 min of glucose deprivation, only 15–20% of the total $V_0$ complexes were assembled with $V_1$. Disassembly was fully reversible; readdition of glucose for as little as five min resulted in quantitative reassembly of $V_1$ with $V_0$, to the same level as that seen before disassembly. Neither disassembly nor reassembly required new protein synthesis, because both occurred equally efficiently in the presence of 100 μg/ml cycloheximide. Changes in assembly state were seen on both short-term time and long-term time scales. Cells grown overnight in a poor carbon source such as raffinose or glycerol–ethanol contained a higher proportion of dissociated $V_1$ and $V_0$ sectors than glucose-grown cells and these dissociated complexes remained competent for reassociation upon glucose readdition [22]. Similarly, cells that were allowed to deplete the glucose in the medium during growth also contained a higher proportion of disassembled V-ATPase after glucose depletion [24], suggesting that shifts in the V-ATPase assembly state occur during normal cell growth and are not being artificially induced by abrupt shifts in carbon source. The situation in tobacco hornworm (*Manduca sexta*) midgut was remarkably similar to that in yeast. In *M. sexta*, disassembly was induced at a developmental stage characterized by cessation of feeding (molting) and could also be induced by starvation of intermolt larvae [23,25]. Refeeding of starved intermolt larvae recruited $V_1$ sectors back to the membrane [25]. In both insects and yeast, disassembly of the V-ATPase resulted in downregulation of ATP hydrolysis and proton pumping at the membrane and reassembly restored these activities [22,23,25].

Our current picture of the reversible disassembly of V-ATPases is shown in Fig. 2. Given that we have never seen complete assembly of V-ATPases in any yeast strain containing a catalytically competent V-ATPase, we hypothesize that the assembled $V_1V_0$ complexes exist in dynamic equilibrium with free $V_1$ and $V_0$ sectors and that changes in carbon source shift this equilibrium to give the final outcome of disassembly or reassembly. One way of looking at this equilibrium is to envision all V-ATPase complexes as cycling between the assembled and disassembled states at a rate characteristic of a given carbon source. In this model, a shift from glucose to a poorer carbon source would cause the enzyme to spend more time in the disassembled state, which could be achieved by either 'trapping' $V_1$ for an extended time in the cytosol or altering $V_0$ such that reassembly with $V_1$ is slowed down.

Regardless of the precise mechanism, disassembly and reassembly of V-ATPases in response to carbon source changes requires that yeast cells be able to rapidly and efficiently communicate a change in extracellular nutrients to V-ATPase complexes present on internal membranes. It is still not clear how such a signal is transmitted to the V-ATPase. Adjustments to the carbon source have been extensively studied in yeast cells [26] and we examined the effects of a number of mutants known to alter glucose-dependent responses on disassembly and reassembly of the yeast V-ATPase [24]. Mutants defective in the ras-cyclic AMP pathway, the Snf1 protein kinase and protein kinase C showed a normal response of the V-ATPase to changes in glucose concentration, suggesting that reversible disassembly of the V-ATPase does not require any of these established signal transduction pathways. In fact, most glucose-dependent signalling pathways in yeast are known to sense the cellular levels of glucose-6-phosphate, but accumulation of glucose-6-phosphate was insufficient to maintain assembly or induce reassembly of the yeast V-ATPase [24]. These results suggest that the V-ATPase may sense the cellular levels of glucose in an unconventional manner that requires metabolism of glucose beyond formation of glucose-

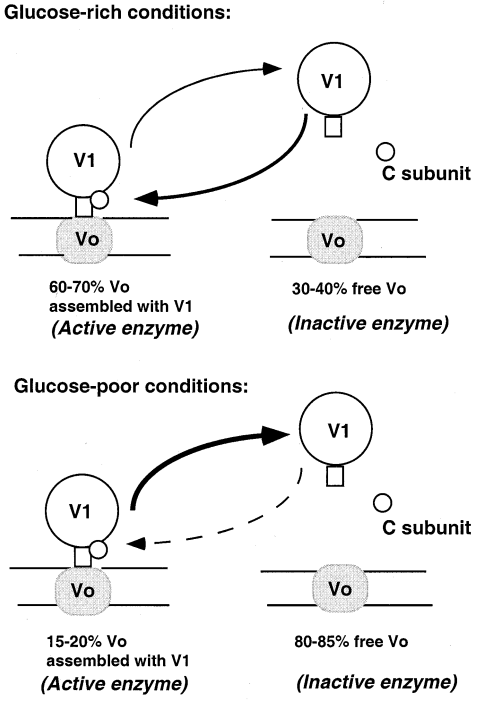


Fig. 2. Dynamic equilibrium between assembled $V_1V_0$ complexes and free $V_1$ and $V_0$ sectors. The $V_1$ sector is shown as constantly cycling between a membrane bound state, where it is structurally and functionally coupled to the $V_0$ sector and able to drive proton pumping, and an inactive, cytoplasmic state. The extent of assembly of $V_1$ with $V_0$ is regulated by glucose and the shift toward disassembly seen in glucose-poor conditions may be achieved by promoting release of $V_1$ from the membrane, retaining it in the cytosol or inhibiting its rebinding to $V_0$ at the membrane.

6-phosphate. One possibility is that the ATPase, which has multiple nucleotide binding sites, responds directly to nucleotide levels in the cell, but the data suggest that nucleotide levels alone are probably not sufficient to establish the V-ATPase assembly state [24]. The catalytic activity of the V-ATPase itself is essential for disassembly of the enzyme; a mutant V-ATPase that was assembly-competent but inactive in both ATP hydrolysis and proton transport was completely unable to disassemble in response to glucose deprivation and was hyperassembled relative to the wild-type enzyme in the presence of glucose [24]. Nucleotide binding without hydrolysis was insufficient to allow disassembly upon glucose deprivation [27]. Earlier experiments on in vitro disassembly of the V-ATPase had also suggested a requirement for catalytic activity in the enzyme [28].

## 3. Structural and functional implications V-ATPase disassembly

The ability of the V-ATPase to switch between assembled and disassembled states has a number of structural implications for the enzyme. Paradoxically, the enzyme must have sufficient intersubunit contacts to communicate a conformation change generated by ATP hydrolysis from nucleotide binding subunits in $V_1$ to proton pore subunits in $V_0$, but at the same time, be able to release these intersubunit contacts rapidly in response to changes in carbon source. Current structural models of the F-ATPases propose that the enzyme has two stalks, which act as a rotor and stator during catalysis (reviewed in [29]). Low resolution structures of V-ATPases indicate that they have at least two, and perhaps three, stalks that must be broken in order for disassembly to occur [11,12,15]. If the catalytic mechanisms of V- and F-ATPases are indeed fundamentally similar, then one of the stalks in the V-ATPase may act as a rotor, which makes alternating contacts with the three catalytic subunits during catalysis. In this context, catalysis may be required for disassembly of the V-ATPase because rotation requires a repetitive release of intersubunit contacts in the rotor stalk and thus 'loosens' this stalk, making it more susceptible to disassembly. One problem with this hypothesis is that it appears that the $F_1$ stalk subunits that form the rotor rotate together with the $F_0$ proteolipid subunits during catalysis [43]. Similar interactions between the $V_1$ rotor subunits and the $V_0$ proteolipid subunits would have to be broken for V-ATPase disassembly to occur. Models for rotation of F-ATPases also require a stable stator connecting $F_1$ and $F_0$ for rotational catalysis to be productive [29]. The existence of reversible disassembly in the V-ATPases requires a less stable stator in these enzymes and suggests that the stalk or stalks that play the role of the stator may be the target of structural changes resulting in disassembly.

Unfortunately, there has been no conclusive assignment of specific V-ATPase subunits to the stalks visualized by electron microscopy, although this is an area of active research [15]. Two of the intermediate size subunits, the D and E subunits, have an extended α-helical structure that resembles that of the $F_1$ γ subunit, the central component of the $F_1$ rotor, and it is not clear which of these might play a similar role to γ in the V-ATPases [7,30]. Genetic and physical interactions have been detected between the D and F subunits and between the E and G subunits [31], and the G subunit exhibits limited homology to the b subunit of F-ATPases, which appears to form part of the stator [7,32]. This forms the basis of the assignments of subunits to the two stalks shown in Fig. 1, but it is still speculative. In addition, the yeast 100-kDa $V_0$ subunit, Vph1p, which has no homolog in F-ATPases, was recently proposed to form part of the connection between the $V_1$ and $V_0$ sectors after it was shown to be responsive to conformational changes in the nucleotide-binding subunits [33]. Finally, the C and H subunits, which also have no homologs in F-type ATPases, are attractive targets for regulation of V-ATPases. Based on the phenotypes of their respective deletion mutants in yeast, both subunits appear to be essential for stable association of $V_1$ with $V_0$ but not for the structure of the $V_1$ and $V_0$ sectors [19,34,35]. Clearly, it is difficult to speculate about which subunit is the target of glucose-induced structural changes at present, but as the positions and functional roles of the individual subunits are elucidated, it should become easier to make testable predictions.

As another route to understanding the structural basis of disassembly, cytosolic $V_1$ complexes have been isolated from both *M. sexta* and yeast cells subjected to conditions that promote disassembly of the V-ATPase ([25], Parra et al., in preparation). The complexes isolated from *M. sexta* contain all of the $V_1$ subunits except possibly subunit H, which has not been conclusively identified in this organism [25,36]. The C subunit also appears to be present at sub-stoichiometric amounts [36]. The yeast $V_1$ complexes completely lack subunit C but contain all of the other $V_1$ subunits including subunit H (Parra et al., in preparation). (Earlier immunoprecipitation studies on the yeast enzyme had indicated that the C subunit appeared to be lost from both the $V_1$ and $V_0$ sectors upon disassembly, but to reassociate with the two sectors during reassembly [22].) As predicted from in vitro studies, $V_1$ sectors from both sources were inactive in $Mg^{2+}$-dependent ATP hydrolysis when released from $V_0$ ([25]; Parra et al., in preparation). This silencing of activity in free $V_1$ sectors is essential in vivo because an active cytosolic $V_1$ complex could rapidly and unproductively deplete cellular ATP stores. It is still unclear how this silencing is achieved, but enzymatic characterization of the purified $V_1$ complexes has provided some insights. Although neither complex shows the physiologically relevant $Mg^{2+}$-dependent ATPase activity, except in the presence of organic solvents, both the *M. sexta* and yeast $V_1$ complexes exhibit a $Ca^{2+}$-dependent ATPase activity in the presence of millimolar $Ca^{2+}$ concentrations ([25]; Parra et al., in preparation). Most free $F_1$ complexes are very active as $Mg^{2+}$-dependent ATP hydrolases in vitro, but a shift from $Mg^{2+}$- to $Ca^{2+}$-dependent activity has been observed in certain soluble $F_1$ complexes [37,38] and attributed to the product inhibition at the catalytic sites, the presence of an inhibitory subunit in the soluble enzyme or the presence of an inhibitory disulfide bond [39,40]. A combination of these factors may also contribute to inactivation of the soluble $V_1$ complexes. Both the *M. sexta* and yeast enzymes exhibit rapid product inhibition of the $Ca^{2+}$-dependent ATPase activity ([25]; Parra et al., in preparation). However, the isolated yeast enzyme does not appear to carry any tightly bound ADP or ATP, so pre-existing nucleotide bound at the catalytic site cannot account for the lack of $Mg^{2+}$-dependent ATPase activity. Experiments with the yeast V-ATPase indicate that the H subunit may have an inhibitory effect on the soluble $V_1$, because $V_1$ complexes isolated from a mutant lacking the H subunit are much more active as $Ca^{2+}$-dependent ATPases

and less susceptible to product inhibition (Parra et al., in preparation). Finally, loss of the C subunit during disassembly of the $V_1$ sector may be critical for the shift in enzymatic behavior of the soluble enzyme. It would not be surprising if a combination of these mechanisms were involved in silencing the soluble $V_1$ sectors, given the wide distribution of free $V_1$ sectors and their potential for damaging effects if left uninhibited.

Reversible disassembly of V-ATPases also has intriguing functional implications. The conditions that generate disassembly and reassembly make physiological sense. Adjustments in the level of assembly of the V-ATPase may be seen as balancing the need to conserve cytoplasmic ATP with the need to maintain intracellular pH homeostasis. Therefore, under conditions where nutrients are limited, the V-ATPase becomes predominantly disassembled and its hydrolytic activity is inhibited, conserving cytoplasmic ATP, but when glucose is restored, the enzyme reassembles and helps to handle the cytosolic acidification that accompanies resumption of active metabolism. What is somewhat surprising about this process is the observation that fairly large pools of apparently inactive free $V_1$ and $V_0$ sectors are maintained by the cell, even during long-term growth in a poor carbon source [22], rather than degraded and resynthesized when conditions improve. One possible reason for maintenance of these pools is that they allow a more rapid reassembly of intact V-ATPases than would be possible through synthesis of new complexes. Another possibility, however, is that the cytoplasmic $V_1$ complexes play an additional cellular role distinct from their role as membrane-bound proton-pumping ATPases. What this role might be is unclear, but a careful analysis of the cytosolic $V_1$ complexes, aimed particularly at assessing whether any new protein–protein interactions are formed with the soluble complexes, could both reveal new roles for the complexes and help uncover how their rate of reassociation with the membrane subunits is regulated. Release of $V_1$ sectors from the membrane during disassembly also opens up the possibility that $V_1$ and $V_0$ sectors could be 'mixed and matched' to give V-ATPase complexes of new properties dependent on the extracellular conditions. Although we were able to show quantitative reassembly of released $V_1$ with $V_0$ sectors after glucose readdition [22,24], there is no way at present to tell whether the disassembled $V_1$ sectors reassemble with the same $V_0$ sectors from which they were released. In yeast cells there appear to be at least two types of $V_0$ sectors, those containing the Vph1p isoform of the 100 kDa subunit and those containing the Stv1p isoform [41], but the much lower abundance of Stv1p makes it hard to distinguish the two types of $V_0$. In other cells, there are also clearly different classes of $V_0$ sectors [42], which may be present in different subcellular locations or generate assembled V-ATPase complexes with different physical properties. If these different $V_0$ sectors compete for disassembled $V_1$ sectors during reassembly, then it is possible that reassembly could result in redistribution of intact V-ATPases or alteration of their activities.

## 4. Conclusion

Much of the work to date on V-ATPases has been aimed toward comparison of these enzymes with the better characterized F-ATPases. It is now clear, however, that there are both significant similarities and significant differences between these two enzyme classes. The regulated instability of V-ATPases is a difference that may ultimately provide insights into the structural and functional coupling of both types of ATPase, but it also suggests that V-ATPases have regulatory features in common with other, very different enzymes that modulate their assembly state and activity in response to extracellular signals, such as trimeric G proteins.

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