Glu-44 in the Amino-terminal \(\alpha\)-Helix of Yeast Vacuolar ATPase E Subunit (Vma4p) Has a Role for \(V_oV_1\) Assembly

Haruko Okamoto-Terry, Kaori Umeki, Mayumi Nakanishi-Matsui, and Masamitsu Futai

From the Department of Biochemistry, Faculty of Pharmaceutical Sciences, Iwate Medical University, Futai Special Laboratory, Yahaba, Iwate 028-3694, Japan

Received for publication, July 31, 2013, and in revised form, October 21, 2013 Published, JBC Papers in Press, November 6, 2013, DOI 10.1074/jbc.M113.506741

Background: The V-ATPase is inactivated by reversible disassembly upon glucose starvation.

Results: Glu-44 of the V-ATPase E subunit was identified by alanine scanning mutagenesis with reduced disassembly under glucose starvation.

Conclusion: Mutations to Glu-44 of the E subunit disrupt normal disassembly of the V-ATPase and affect catalytic activity.

Significance: Interactions with the E subunit in the V-ATPase are important for controlling disassembly and catalytic activity.

The proton (\(H^+\)) pumping vacuolar-type ATPase (V-ATPase) is a rotary enzyme that plays a pivotal role in forming intracellular acidic compartments in eukaryotic cells. In Saccharomyces cerevisiae, the membrane extrinsic catalytic \(V_1\) and the transmembrane proton-pumping \(V_o\) complexes have been shown to reversibly dissociate upon removal of glucose from the medium. However, the basis of this disassembly is largely unknown. In the earlier study, we have found that the amino-terminal \(\alpha\)-helical domain between Lys-33 and Lys-83 of yeast E subunit (Vma4p) in the peripheral stalk of the \(V_1\) complex has a role in glucose-dependent \(V_oV_1\) assembly. Results of alanine-scanning mutagenesis within the domain revealed that the Vma4p Glu-44 is a key residue in \(V_oV_1\) disassembly. Biochemical analysis on Vma4p Glu-44 to Ala, Asn, Asp, and Gln substitutions indicated that Glu-44 has a role in V-ATPase catalysis. These results suggest that Glu-44 is one of the key functional residues for subunit interaction in the V-ATPase stalk complex that allows both efficient rotation catalysis and assembly.

The vacuolar-type ATPase (V-ATPase) hydrolyzes ATP to ADP and phosphate coupled to \(H^+\) transport through rotation of a transmembrane complex. The V-ATPase is expressed ubiquitously in yeast, plant, nematode, insect, and mammalian cells and has essential roles in forming intracellular acidic compartments (1–3). Yeast V-ATPase comprising 14 subunits has a catalytic \(V_1\) \((A, B, C, D, E, F, G, H)\) and a membrane intrinsic proton pumping \(V_o\) complex \((a, c, c', c'', d, e)\) (4–6). The yeast genome contains 13 VMA (vacuolar membrane associated) genes encoding these subunits except for the a subunit that has two isoforms encoded by \(VPH1\) and \(STV1\). Vma null mutants are conditional lethal and do not grow above neutral pH, indicating that the V-ATPase is essential for growth at alkaline pH (5).

The energy generated by the catalytic \(V_1\) complex is coupled to the revolution of the central stalk, the D subunit that in turn rotates the proton transporting \(V_o\) complex. Studies using x-ray crystallography and electron microscopy have predicted that yeast V-ATPase has three peripheral stalks that are supporting the catalytic \(A_3B_3\) hexamer, with each of them formed from a heterodimer of the E and G subunits (7–11). NMR spectroscopy of the 69 amino-terminal residues of the yeast E subunit (12), and crystal structure analyses of the heterotrimeric EGC complexes (Protein Data Bank codes 4DL0 and 4EFA) have shown that the yeast V-ATPase E and G subunits have an amino-terminal right-handed coiled-coil structure that allows intrinsic flexibility to the stalks (8). The structural flexibilities of the peripheral stalks would allow conformational transition between catalytic sites in the \(A_3B_3\) hexamer (13).

Yeast V-ATPase activity is, at least in part, regulated by the availability of glucose in the medium. The C subunit is released from the enzyme within 5 min after removal of glucose, resulting in disassembly of \(V_1\) from the \(V_o\) complex and termination of ATP-dependent proton-pumping activity (5, 14–16). The C subunit and \(V_1\) assemble with the \(V_o\) in a glucose-dependent and reversible manner. Similar disassembly was also reported in Manduca sexta, in which the \(V_1\) complex is released to the cytoplasm during molting at the larval stage (17–20), suggesting that V-ATPase disassembly is regulated by the energy state in a wide range of organisms across the phyla.

Unlike those of yeast, mammalian E and G subunits have a number of isoforms that are unique and specific for the cell/tissue type. The mouse E subunit has two isoforms: E1, specific for testis and the acrosome of spermatozoa; E2, ubiquitously expressed (21, 22). The E subunits are well conserved, and those of mouse and yeast share \(30\%\) amino acid identity. Secondary structure prediction analyses have indicated even higher simi-
larity in their structures (12, 23). We have previously shown that a null mutant of yeast E subunit (Δvma4) (24) expressing E1 or E2 grows at alkaline pH and that the mouse E1 or E2 yeast hybrid V-ATPase transports protons, indicating that the mouse isoforms are functional in yeast (22). However, the Vα and V1 assembly of the hybrid V-ATPase was less dependent on glucose (25). These results suggested that the E subunit, specifically the yeast Vma4p, has an important role in V-ATPase disassembly.

In this study, we have developed an experimental system using the C subunit tagged with GFP that allows us to study the functional domain and/or the amino acid residue(s) of Vma4p for glucose-dependent V-ATPase disassembly (see Fig. 1A). The Glu-44 and its vicinity in the amino-terminal α-helical domain of Vma4p were identified as having a key role in V-ATPase disassembly and catalysis.

**EXPERIMENTAL PROCEDURES**

**Materials and Strains**—The MATa yeast, in which a GFP (S65T) gene was inserted at the C-terminal end of VMA5, generated by O’Shea and Weissman (26), and the MATα (BY4742) vma4 knockout mutant (YOR332W) were purchased from Invitrogen and Open Biosystems, respectively. Cells transformed with the yeast expression vector pKT10 were selected and maintained on SD-uracil medium. The strains used in this study were grown in yeast extract-peptone-2% dextrose (YPD) medium buffered to pH 5.0 with 50 mM sodium succinate/50 mM sodium succinate (YPD, pH 5.0), and/or YPD medium buffered to pH 7.5 with 50 mM sodium phosphate (YPD, pH 7.5). Reagents for the growth medium were obtained from BD Biosciences.

**Reagents**—Reagents for molecular biology were purchased from Takara Bio (Kyoto, Japan), New England Biolabs, Sigma-Aldrich, and Nacalai Tesque Co. (Osaka, Japan). Bafilomycin A1 was purchased from Wako Chemicals Co. Pyruvate kinase, phosphoenol pyruvate, and lactate dehydrogenases were obtained from Roche Applied Science. Total yeast protein extract was obtained by lysing cells using cracking buffer (8 M urea, 5% sodium dodecyl sulfate, 40 mM Tris-Cl, pH 6.8, 0.1 mM EDTA, 0.4 mg/ml bromphenol blue). Monoclonal antibodies for anti-Myc epitope (9E10) and the anti-V-ATPase subunit A antibody conjugated with protein A-Sepharose CL-4B beads (GE Healthcare) for immunoprecipitation of VαV1 by the method described (25).

Vacuolar membranes used for biochemical studies were isolated from the cells by the method described previously (27). Membranes were treated with or without 100 nM Bafilomycin A1 for 1 h prior to the assay in 150 mM KCl and 20 mM HEPES, pH 7.0, with KOH. Bafilomycin A1-sensitive ATPase activity was determined at 30 °C by a NADH-coupled assay system (28). The protein concentrations were determined by the Bradford method (29).

**RESULTS AND DISCUSSION**

Reduced Disassembly in Mouse E1 Yeast Hybrid V-ATPase—We have developed a system in which the C subunit is tagged with GFP (C-GFP) that enables us to observe the disassembly of V-ATPase in real time (Fig. 1A). A yeast Δvma4::C-GFP cell, lacking the E subunit (VMA4) gene and expressing the C subunit tagged with GFP, was generated by a genetic cross and was transformed with a yeast expression vector carrying the E subunit gene.

The resulting transformants expressing the cDNA for Vma4p or mouse E1 or E2 were all able to grow on glucose medium (pH 7.5), and C-GFP fluorescence was observed in the vacuolar membrane (Fig. 1B), indicating that both E1 and E2 have restored the function and localization of the V-ATPase similarly to restoration by Vma4p. When glucose was removed from the medium, C-GFP was observed in the cytoplasm of the cells expressing Vma4p (Fig. 1B, VMA4). In contrast, C-GFP was found primarily in the vacuolar membrane of the transformants expressing E1 and E2 (Fig. 1B, E1 and E2), indicating that the hybrid V-ATPases are still assembled after removal of glucose. This result is consistent with our previous immunoprecipitation data showing that the Vα, V1 complexes of mouse E1 yeast hybrid V-ATPase are mostly associated after removal of glucose (25). These results indicate that our newly developed experimental system allows us to analyze the critical domain of VMA4 for VαV1 disassembly in real time without having to perform time-consuming immunochemical procedures.
**FIGURE 1.** V-ATPase V, V complex disassembly observation by C-GFP. A, a schematic illustration of V-ATPase V, complex assembly to the V, in the vacuolar membrane and the V, release to the cytoplasm in the presence and absence of glucose. GFP fused to the C subunit in the V, complex is in the vacuolar membrane when V,V is associated and is dissociated from V, in the absence of glucose. B, localization of C-GFP in yeast cells expressing mouse E1 or E2 yeast hybrid V-ATPase. Δvma-4::C-GFP cells transformed with recombinant plasmids carrying mouse E1 (E1), E2 (E2), or yeast E subunit (VMA4) or the empty vector (vec) were grown in SD-ura medium for 36 h. Cells were washed and incubated in a fresh medium with (Glc) or without glucose (−) for 20 min prior to observation under a confocal microscope. A solid arrowhead indicates typical localization of C-GFP in mid-slice view of the vacuolar membrane. DIC, differential interference contrast microscopy.

**FIGURE 2.** Localization of C-GFP in yeast cells expressing V-ATPases with mouse/yeast chimeric E subunits. A, Δvma-4::C-GFP cells expressing mouse E1/Vma4p chimeric subunits, E1/VMA4−1, E1/VMA4−2, and E1/VMA4−5 (constructs described in Ref. 25) were grown in SD-ura medium and treated as above. C-GFP localization is shown in the panels labeled GFP. Light field images together with both images overlaid (Merged). A double arrowhead indicates typical localization of C-GFP in a domed top view of the vacuolar membrane. B, a ClustalW alignment of the amino-terminal sequences of mouse E1, E2, and Vma4p is shown. Conserved and non-conserved residues are indicated with asterisks and bars, respectively. The 34 non-conserved residues between Lys-33 and Lys-83 of Vma4p are shown in red.
The Amino-terminal Domain of Mouse E1 Affects V-ATPase Disassembly—Unlike the authentic yeast V-ATPase, the V-ATPase containing the mouse E1 yeast hybrid does not readily disassemble upon removal of glucose from the medium (Fig. 1B). To determine which domain of E1 affects the disassembly, we have tested a series of previously constructed plasmids (25) that express E1/Vma4p chimeric E subunits and examined their assembly properties. First, a chimeric E subunit in which Met-1 to Lys-33 of Vma4p was replaced by the corresponding residues of E1 (E1/VMA4–1) was expressed in /H9004 vma4::C-GFP cells. These cells showed the vacuolar localization of C-GFP only in the presence of glucose (Fig. 2A), indicating that this region does not affect the glucose-dependent assembly of yeast V-ATPase. Second, the region between Met-1 and Lys-83 of Vma4p replaced by the corresponding residues of E1 (E1/VMA4–2) was expressed in the /H9004 vma4::C-GFP cells. These cells had C-GFP fluorescence in the vacuolar membrane irrespective of the presence or absence of glucose. Because E1/VMA4–1 does not affect the glucose-dependent assembly of V-ATPase, the region between Lys-33 and Lys-83 of Vma4p is likely to be affecting the glucose-dependent C-GFP localization. As expected, cells expressing a chimeric E subunit with the E1 residues corresponding Lys-33 to Lys-83 of Vma4p showed the vacuolar localization of C-GFP irrespective of glucose (Fig. 2A). These results indicate that the amino-terminal region of Vma4p between Lys-33 and Lys-83 contains residue(s) responsible for glucose-dependent disassembly.

Glu-44 of Vma4p Is Essential for Glucose-dependent Disassembly—The above data indicated that the 34 residues between Lys-33 and Lys-83 of Vma4p that are not conserved in E1 (Fig. 2B; indicated in red) may have a role in glucose-dependent assembly of V-ATPase. To test this hypothesis, these 34 residues were substituted one by one with Ala, so that we could change each functional moiety without drastically interfering with the helical property of this domain (30).

The Δvma4Δ-C-GFP cells expressing each mutant Vma4p restored the defective growth of Δvma4Δ on alkaline pH, indicating that all 34 mutants form an active V-ATPase (data not shown). We then tested these substitution mutants for the subcellular localization of C-GFP with or without glucose. The Glu-44 to Ala mutant showed primarily vacuolar membrane localization of C-GFP irrespective of the presence of glucose (supplemental Fig. 1). In addition, Ala substitution mutants of
Glu-54, Gly-60, and Asn-61 showed partial vacuolar membrane localization of C-GFP (supplemental Fig. 1). The latter three mutations resulted in C-GFP localization to both the cytoplasm and vacuolar membrane irrespective of the presence of glucose. These results indicate that Ala substitution at these three positions is generally affecting V-ATPase assembly, whereas the Glu-44 to Ala substitution results in reduced V-ATPase disassembly by glucose depletion. We therefore further investigated the role of Glu-44 in glucose-dependent disassembly.

Effects of Glu-44 to Ala, Asn, Asp, or Gln Substitutions on V-ATPase Disassembly—As shown in the amino acid alignment (Fig. 2B), the mouse E subunits are shorter than Vma4p by five residues; thus, the Vma4p Glu-44 corresponds to Asn-39 in E1 and E2. Because the mouse E1 yeast hybrid V-ATPase does not disassemble upon removal of glucose, Vma4p with a Glu-44 to Asn substitution results in reduced V-ATPase disassembly by glucose depletion. We therefore further investigated the role of Glu-44 in glucose-dependent disassembly.

Effects of Glu-44 to Ala, Asn, Asp, or Gln Substitutions on V-ATPase Disassembly—As shown in the amino acid alignment (Fig. 2B), the mouse E subunits are shorter than Vma4p by five residues; thus, the Vma4p Glu-44 corresponds to Asn-39 in E1 and E2. Because the mouse E1 yeast hybrid V-ATPase does not disassemble upon removal of glucose, Vma4p with a Glu-44 to Asn substitution may be less sensitive to glucose. In addition, the carboxyl moiety and/or the length of side chain of the residue at position 44 may affect disassembly. Vma4p with Glu-44 to Asn, Asp, and Gln substitution mutants were therefore constructed based on these considerations.

These substitution mutants all complemented the Δvma4 phenotype and growth at alkaline pH was indistinguishable from that of wild-type (Fig. 3A), indicating that the Vma4p Glu-44 substitutions are functional. Furthermore, cells transformed with the wild-type VMA4 gene and with the Glu-44 substitutions, all contained essentially the same amount of Vma4p (Fig. 3B), suggesting that a comparable amount of V-ATPase is expressed in these cells.

Similarly to the cells expressing Vma4p with Glu-44 substitution to Ala (E44A), the Asn (E44N), Asp (E44D), and Glu (E44Q) substitutions all showed vacuolar C-GFP localization in the absence of glucose (Fig. 4A), whereas C-GFP was observed mostly in the cytoplasm in the wild-type (Glu-44). These results suggest that a considerable amount of the Vo and V1 complexes are still associated in these Glu-44 substitution mutants.

To examine the VoV1 association, protoplasts were prepared from the cells expressing wild-type Vma4p, and the E44A, E44N, E44D, and E44Q substitutions and were incubated with or without glucose for 20 min prior to an osmotic lysis of the plasma membrane. The VoV1 complex was immuno-precipitated by an anti-A subunit antibody and the co-precipitation of Vo subunit α was examined using an anti-Vph1p specific antibody (Fig. 4B). Vph1p was detected in all cells incubated with glucose. In agreement with the microscopic observations of the subcellular localization of C-GFP (Fig. 4A, no sugar), <10% of the Vph1p was detectable by densitometry in the protoplasts expressing wild-type Vma4p without glucose compared with those with glucose. In contrast, >30% of the Vph1p was still detected in the E44A, E44N, E44D, and E44Q substitution mutant cells incubated without glucose compared with those with (Fig. 4B). These results indicated that VoV1 is still mostly associated in the mutants under this condition.
To examine whether these substitutions resulted in a change of V<sub>o</sub>V<sub>1</sub> disassembly kinetics, we performed a real time observation of the vacuolar localization of C-GFP after removal of glucose over 60 min. We have used cells in the mid-logarithmic phase for this analysis. It should be noted that these cells have ~70% assembled V-ATPase that is close to previous biochemical observations (16). To study disassembly kinetics, these cells with C-GFP localized in the vacuolar membrane in SD medium were followed after removal of glucose.

Consistent with the previous results (14, 31), the vacuolar C-GFP localization of typical wild-type cells became mostly cytoplasmic within 6 min after the removal of glucose with some C-GFP remaining in the vacuolar membrane (Fig. 5A; 6 min, closed arrowhead). In the majority of wild-type cells, the C-GFP became evenly distributed across the cytoplasm by 10 min (Fig. 5A; 10 min, open arrowhead) indicating the dissociation of V<sub>1</sub> and V<sub>o</sub>. The time course of V<sub>1</sub> dissociation from V<sub>o</sub> within (using ClustalW alignments). It is interesting to note that responding position in Vma4p with fungi, plants, and animals specific role for assembly. These residues are found at the corresponding position in Vma4p with fungi, plants, and animals (Supplemental Fig. 2). The alignment of Vma4p and mouse E subunits indicated that the Asn residue is conserved in the Ascomycota, Glu-44 is conserved in the Pezizomycotina, and the Gln residue is conserved within the Schizosaccharomyces except at the corresponding position (Supplemental Fig. 2). The alignment of Vma4p and mouse E subunits indicated that the Asn residue is conserved at the corresponding position (Fig. 2B) within the whole of Animalia and Plantae.

Effects of Glu-44 Substitution on ATPase Activity—The Glu-44 substitutions may have affected the Vma4p conformation and made the V-ATPase harder to disassemble. However, it is difficult to further assess the effects of amino acid substitutions on the disassembly kinetics.

The E subunit serves as a part of three stators those support the A<sub>B</sub><sub>D</sub> catalytic hexamer to the V<sub>o</sub> sector. These stators have been shown to have flexible arms (8), and most likely regulate the ATP binding, chemical reaction, or release of ADP/Pi in a sequence of rotational catalysis. Therefore, conformational changes of the E subunit may affect stator structure leading to the indirect effects on V-ATPase catalysis. Thus, it became of interest to test the effects of E subunit mutations on V-ATPase catalysis.

As expected, the substitutions of Glu-44 all affected the activation energy for ATPase catalysis (Fig. 6A). It is not immediately obvious how substitutions in Glu-44 of Vma4p, that is a
These results may be indicating that the Vma4p Glu-44 substitutions were caused an increase in the activation energy for V-ATPase catalysis. The slope of Arrhenius plots of E44D was closely similar to that of WT (Fig. 6B). Thus, the carboxyl moiety of Glu-44 (WT) may contribute to lower the enthalpy for catalysis. The increase in enthalpy for catalysis in E4N and E44Q mutants may reflect their gain of rigidity in the peripheral stalks, leading to a reduced V-ATPase disassembly upon glucose removal.

WT and E44D, those with a carboxyl moiety, had similar activation energy for V-ATPase catalysis. The slope of Arrhenius plot of E44D was closely similar to that of WT (Fig. 6A). However, E44D showed higher rate of V-ATPase activity than WT between 20 and 34 °C. These results suggest that the rotational catalysis rate of V-ATPase with E44D substitution is faster than that of WT.

It is worth noting that E44A also showed higher rate of V-ATPase activity than WT and the $V_{\text{max}}$ at 30 °C was similar to that of E44D. These results indicated that mutants with shorter amino acid side chain had similar catalysis. The increase of $V_{\text{max}}$ rate with E44D and E44A mutants may be due to the altered conformation of the complex. This change in conformation may have resulted in a gain-of-interactive interaction between subunits that likely lead to the reduced V-ATPase disassembly in those E44D and E44A upon glucose removal.

The transition state thermodynamics parameters showed that the substitutions have a relatively small effect on free energy ($\Delta G^{\ddagger}$) obtained by catalysis (Fig. 6, B and C), whereas enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) were increased by the substitutions (Fig. 6, B and C). The results suggest that these substitutions caused an increase in the activation energy for V-ATPase catalysis.

Thermodynamic analysis has shown that the increase in enthalpy ($\Delta H^\ddagger$) and entropy ($\Delta S^\ddagger$) of the Vma4p E44D mutant were $\sim$20 kJ/mol. The values for the E44N and E44Q substitutions were $\sim$60 kJ/mol and those of E44A were $\sim$120 kJ/mol. These results may be indicating that the Vma4p Glu-44 substitutions have rendered the V-ATPase with extra bonds (i.e. electrostatic or hydrophobic interactions) between subunits giving it a stronger structural entity than that of wild-type. Recently, x-ray crystallography analysis of the heterotrimeric EGC complexes has identified two hinge regions “bulge” and “skip” in the E subunit (8) that are flanked by the right-handed coiled-coil helix domain. These hinges had been suggested to contribute to the conformational flexibility that may be also important for priming the V-ATPase complex for rapid disassembly in response to glucose availability (8, 34).

Similarly to Vma4p, Vma10p (yeast G subunit) may also have a role in glucose-dependent $V_o/V_i$ disassembly as the Arg-25 to Ala or Leu substitutions affect the assembly (35). The Arg-25 and Glu-44 residues may be interacting, however, the coordinates of the carboxyl moiety of the Glu-44 residue in the predicted structures suggest that such an interaction is unlikely to be formed (Fig. 7). Nevertheless, Vma4p was shown to interact with the C, G, and H subunits (36–39). Therefore, Vma4p Glu-44 may be important for interactions with other stalk subunits.

Conclusions—Gain of function by domain exchange and alanine scan mutagenesis of the E subunit in the peripheral stalk of V-ATPase indicated that the stalk subunit interaction with V-ATPase is important for catalytic activity and disassembly in response to glucose starvation. In addition, the alanine scan analysis identified Glu-44 as the residue that may prevent the formation of a strongly-held coiled coil that allow V-ATPase disassembly. We propose that the Glu-44 provide Vma4p a role in glucose-dependent VoV1 disassembly as the Arg-25 to Ala or Leu substitutions affect the assembly (8).

**TABLE 1**

The V-ATPase activity and catalysis of the Glu-44 substitution mutants

| Substitutions | $V_{\text{max}}$ (μmol/min/mg vacuolar protein) | $K_m$ (μM ATP) | Activation energy for catalysis (kJ/mol) |
|---------------|-----------------------------------------------|----------------|----------------------------------------|
| None (WT)     | 40 ($\pm$13)                                  | 190 ($\pm$63)  | 31                                     |
| Alanine (A)   | 95 ($\pm$41)                                  | 119 ($\pm$61)  | 155                                    |
| Asparagine (N)| 29 ($\pm$5)                                   | 73 ($\pm$32)   | 90                                     |
| Aspartate (D) | 67 ($\pm$8)                                   | 58 ($\pm$15)   | 49                                     |
| Glutamine (Q) | 44 ($\pm$32)                                  | 85 ($\pm$37)   | 95                                     |

These results suggest that an interaction is unlikely to be formed (Fig. 7). Nevertheless, Vma4p was shown to interact with the C, G, and H subunits (36–39). Therefore, Vma4p Glu-44 may be important for interactions with other stalk subunits.
structural flexibility that allows the V-ATPase catalysis with low energy cost. Further structural analyses of the Vma4p with substitutions may provide further insight into the critical role of the E subunit in the V-ATPase activity.

Acknowledgments—We thank Mitsuru Endo and Ai Kakizoe for excellent technical assistance. The polyclonal antibody used to detect Vph1p was a kind gift from Dr. Ryogo Hirata at the RIKEN Institute.

REFERENCES

1. Futai, M., Nakashiki-Matsui, M., Okamoto, H., Sekiya, M., and Nakamoto, R. K. (2012) Rotational catalysis in proton pumping ATPases: From E. coli F-ATPase to mammalian V-ATPase. Biochim. Biophys. Acta 1817, 1711–1721
2. Marshansky, V., and Futai, M. (2008) The V-type H+-ATPase in vesicular trafficking: targeting, regulation, and function. Curr. Opin. Cell Biol. 20, 415–426
3. Okamoto, H., Futai, M. (2013) Vacular-Type ATPases in Animal and Plant Cells in Encyclopedia of Biophysics (Roberts, G. C. K., ed) pp. 2719–2724; Springer-Verlag Berlin, Heidelberg, Berlin
4. Forgac, M. (2007) Vacular ATPases: rotary proton pumps in physiology and pathophysiology. Nat. Rev. Mol. Cell Biol. 8, 917–929
5. Kane, P. M. (2006) The where, when, and how of organelle acidification by the yeast vacuolar H+-ATPase. Microbiol. Mol. Biol. Rev. 70, 177–191
6. Compton, M. A., Graham, L. A., and Stevens, T. H. (2006) Vma9p (subunit e) is an integral membrane V$_v$ subunit of the yeast V-ATPase. J. Biol. Chem. 281, 15312–15319
7. Diepholz, M., Venzke, D., Prinz, S., Battise, C, Flörchinger, B., Rössle, M., Svergun, D. I., Böttcher, B., and Féthière, J. (2008) A different conformation for EGC stator subcomplex in solution and in the assembled yeast V-ATPase: possible implications for regulatory disassembly. Structure 16, 1789–1798
8. Oot, R. A., Huang, L. S., Berry, E. A., and Wilkens, S. (2012) Crystal Structure of the Yeast Vacuolar ATPase Heterotrimeric EGC(head) Peripheral Stalk Complex. Structure 20, 1881–1892
9. Oot, R. A., and Wilkens, S. (2010) Domain characterization and interaction of the yeast vacuolar ATPase subunit C with the peripheral stator stalk subunits E and G. J. Biol. Chem. 285, 24654–24664
10. Oot, R. A., and Wilkens, S. (2012) Subunit interactions at the V$_v$/V$_c$ interface in yeast vacuolar ATPase. J. Biol. Chem. 287, 13396–13406
11. Zhang, Z., Zheng, Y., Mazon, H., Milgrom, E., Kish-Trier, E., Heck, A. J., Kane, P. M., and Wilkens, S. (2008) Structure of the yeast vacuolar ATPase. J. Biol. Chem. 283, 35983–35995
12. Rishikesan, S., Thaker, Y. R., and Grüber, G. (2011) NMR solution structure of subunit E (fragment E(1–69)) of the V-ATPase determined by solution NMR spectroscopy. J. Bioenerg. Biophys. Acta 1777, 1370–1377
13. Huh, W. K., Falvo, J. V., Gerke, L. C., Carroll, A. S., Howson, R. W., Weissman, J. S., and O’Shea, E. K. (2003) Global analysis of protein localization in budding yeast. Nature 425, 686–691
14. Takeshige, K., Baba, M., Tsuibo, S., Noda, T., and Ohsumi, Y. (1992) Autophagy in yeast demonstrated with protease-deficient mutants and conditions for its induction. J. Cell Biol. 119, 301–311
15. Kane, P. M. (2005) Close-up and genomic views of the yeast vacuolar H+-ATPase. J. Bioenerg. Biomembr. 37, 399–403
16. Dechant, R., Binda, M., Lee, S. S., Pelet, S., Winderickx, J., and Peter, M. (2010) Cytosolic pH is a second messenger for glucose and regulates the PKA pathway through V-ATPase. EMBO J. 29, 2515–2526
17. Schnitzer, D., Sander, T., Goldlack, D., and Dietz, K. J. (2011) The cellular energization state affects peripheral stalk stability of plant vacuolar H+-ATPase and impairs vacuolar acidification. Plant Cell Physiol. 52, 946–956
18. Blaber, M., Zhang, X. I., and Matthews, B. W. (1993) Structural basis of amino acid α helix propensity. Science 260, 1637–1640
19. Kane, P. M. (2005) Close-up and genomic views of the yeast vacuolar H+-ATPase. J. Bioenerg. Biomembr. 37, 399–403
20. Dechant, R., Binda, M., Lee, S. S., Pelet, S., Winderickx, J., and Peter, M. (2010) Cytosolic pH is a second messenger for glucose and regulates the PKA pathway through V-ATPase. EMBO J. 29, 2515–2526
21. Schnitzer, D., Sander, T., Goldlack, D., and Dietz, K. J. (2011) The cellular energization state affects peripheral stalk stability of plant vacuolar H+-ATPase and impairs vacuolar acidification. Plant Cell Physiol. 52, 946–956
22. Stewart, A. G., and Stock, D. (2012) Priming a molecular motor for disassembly. Structure 20, 1799–1800
23. Charsky, C. M., Schumann, N. J., and Kane, P. M. (2000) Mutational analysis of subunit G (Vma10p) of the yeast vacuolar H+-ATPase. J. Biol. Chem. 275, 37232–37239
24. Féthière, J., Venzke, D., Diepholz, M., Seybert, A., Gerlof, A., Gentzel, M., Wilm, M., and Böttcher, B. (2004) Building the stator of the yeast vacuolar ATPase: specific interaction between subunits E and G. J. Biol. Chem. 279, 40670–40676
25. Flannery, A. R., and Stevens, T. H. (2008) Functional characterization of the N-terminal domain of subunit H (Vma13p) of the yeast vacuolar ATPase. J. Biol. Chem. 283, 29099–29108
26. Jones, R. P., Durose, L. J., Findlay, J. B., and Harrison, M. A. (2005) Defined sites of interaction between subunits E (Vma4p), C (Vma5p), and G (Vma10p) within the stator structure of the vacuolar H+-ATPase. Biochemistry 44, 3933–3941
27. Lu, M., Vergara, S., Zhang, L., Holliday, L. S., Aris, J., and Gluck, S. L. (2002) The amino-terminal domain of the E subunit of vacuolar H+-ATPase (V-ATPase) interacts with the H subunit and is required for V-ATPase function. J. Biol. Chem. 277, 38409–38415