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Hidden Secrets of Proton Pumping - The Resolution Revolution Enlightens V-ATPases

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Roh *et al.* (2018) present a high-resolution cryo-EM structure of the nanodisc-reconstituted yeast V_o proton channel that provides important new insights into subunit arrangements and the proton translocation pathway in V-type ATPases.

The rotary ATPase family comprises the mitochondrial ATP synthases (F-ATPases), vacuolar ATPases (V-ATPases) and archaeal ATPases (A-ATPases), all of which either utilise a proton gradient to produce ATP or utilise ATP hydrolysis to generate a proton gradient (Muench *et al*., 2011). These sophisticated enzymes are crucial players in many vital cellular processes and mutations affecting their functions cause a large variety of human diseases. An important member of this family, the V-ATPase, consists of an ATP-hydrolysing V_1 domain composed of the subunits A_3, B_3, E_3, F, G_3, C, H and D that are linked to a membrane-bound V_o domain consisting of a variable sized ring of *c* subunits, subunits *a* and *e* and the recently identified subunit *f* (Fig. 1). Through ATP hydrolysis in the V_1 domain, the central rotor axle is rotated by ~120° per ATP used. The resulting torque drives the rotation of the linked *c*-ring, against subunit *a*, resulting in proton translocation across the membrane and the acidification of a subcellular compartment. For many years the fundamental mechanisms of torque generation and ATP handling have been established with crystal structures of the F_1, V_1 and A_1 domains revealing the structural rearrangements which accompany ATP binding and hydrolysis (Suzuki *et al*., 2016). However, due to limitations in resolution the exact pathway of proton transport within the membrane-bound V_o domain has remained largely elusive. In this issue of *Molecular Cell* Roh *et al.* present a high-resolution cryo-EM structure of the yeast V_o proton channel that sheds exciting new light on the mechanism of proton pumping by V-ATPases.

The first single particle cryo-EM reconstruction of the V-ATPase was published in 2009 to a modest ~20 Å resolution. However, recent developments in electron microscopy have enabled a more detailed understanding of the proton transport mechanism, including significantly higher resolution structures of both the *Manduca sexta* (~9 Å) and yeast V-ATPase (~7 Å) complex (Rawson *et al*., 2015 & Zhao *et al*., 2015). Other structural studies have made use of the fact that V-ATPases undergo controlled dissociation resulting in V_1 separating from the V_o domain such that ATP hydrolysis and proton transport are silenced, with the individual domains of V_1 and V_o being determined to 6.0 Å and 3.9 Å, respectively (Oot *et al*., 2016, Oot *et al*., 2017 & Mazhab-Jafari *et al*., 2016). As the resolution of these models has increased, our understanding of how ATP hydrolysis is linked to proton movement over the membrane and how the complex resists the backflow of protons after the generation of a membrane potential has been developed. In 2015, a key step forward in understanding the mechanism of proton transport within the *a*-subunit was taken, when it

was shown in both the F- and V-ATPase that two horizontal α-helices flank the *c*-ring facilitating proton transport. This finding was in stark contrast to earlier models, which reported an 8 α-helical bundle with all helices traversing the membrane (Allegretti *et al*., 2015 & Zhao *et al*., 2015).

In their new paper, the Wilkens group have now determined the structure of a lipid nanodisc-reconstituted yeast V-ATPase V_o domain that reveals important new insights into the proton translocation pathways at the interface between the *c*-ring and subunit *a*. The use of a nanodisc to encapsulate the V_o domain is a powerful approach for EM studies as it provides a more "native"-like lipid environment for the domain which is reported to be one of the contributing factors to the improved resolution. Moreover, the study uses an autoinhibited form of V_o, which is an improvement over previous high-resolution structures that were determined in the presence of inhibitors (bafilomycin), making it difficult to ascertain if the *c*-ring/*a*-subunit interface represents an autoinhibited, native or inhibited state (Mazhab-Jafari *et al*., 2016). The resulting EM map clearly shows two negatively charged half-channels with access from the cytoplasmic and luminal side that are responsible for the entrance and exit of protons across the membrane (Fig. 1). These two cavities are separated by an electrostatic barrier in the middle of the membrane formed by positively charged side chains that may ensure that protons travel along the *c*-ring clockwise through the bilayer. While the fully conserved E137 residue on the *c*-subunit is well characterised for its role in the proton translocation mechanism, the higher resolution afforded by this V_o map implicates further residues in the proton transfer mechanism.

Another intriguing aspect of the work by Roh *et al.* is the assignment of the C-terminal domain of the assembly factor Voa1 to an additional density within the *c*-ring. The presence of Voa1 in the native V_o complex was confirmed by mass spectrometry analysis of purified V_o and holo-V_1V_o complexes and by the absence of the corresponding density in a V_o complex purified from Voa1-deficient cells. The Voa1-free V_o complex shows signs of instability, such as enhanced degradation of subunit *a* and partial loss of subunit *d* from the purified complex. Thus, Voa1 is not only involved in assembly, trafficking and quality control of V-ATPases, but at least the C-terminal domain is also important for the integrity of the mature complex.

Overall, the new findings reported by Roh *et al*., will be an important step in our understanding of V-ATPases. As structural biology looks to move towards more native systems for membrane proteins, including nanodiscs, styrene-maleic acid lipid particles (SMALPs) and liposomes, we are starting to generate a better understanding of protein complexes within lipid bilayers. The improved models will drive our understanding of how point mutations cause specific disease phenotypes through changes in regulation, location and function. With improved resolution also comes better models for structure-based inhibitor design. The current compounds that target V-ATPases are often large macrolides which are difficult to produce and show low specificity. However, if we are to selectively target the V-ATPase for therapeutic intervention we must design new compounds that can tackle the subtle differences between specific isoforms and/or species. Although a significant challenge, there are inhibitors, such as Pea Albumin Toxin 1, that show high selectivity towards specific V-ATPase isoforms (Rahioui *et al*., 2014). As the V-ATPase and wider

rotary ATPase family keeps providing new surprises the future for therapeutic development and understanding disease phenotypes will be an interesting ride.

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Figure Legend

Figure 1. The Yeast V_o domain situated within the lipid bilayer. The V_0 domain situated within the membrane with the subunits *c*, *d*, *a* and *e* shown in green, orange, grey and red, respectively. The blue sphere represents a proton in the cytoplasmic cavity, before being loaded onto the *c*-ring. The model is based on the deposited V_o structure 5tj5 (Mazhab-Jafari *et al.*, 2016).