The Molecular V Brake

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Vacuolar H^+-ATPases (V-ATPases) are a class of rotary ATPase that utilize the energy from ATP hydrolysis to pump protons against a membrane potential. This process serves to acidify intracellular compartments and generate transmembrane electrochemical potential gradients in eukaryotic cells [1]. V-ATPases are composed of two rotary motors: a soluble V_1 that hydrolyses ATP and a transmembrane V_O that pumps protons (Fig. 1). These motors are coupled together by a central rotor and three peripheral stalks. This arrangement facilitates the transfer of torque from the V_1 to the V_O motor [1]. The activity of the complex must be regulated to prevent ATP hydrolysis when cellular ATP concentrations are low or when acidification is not needed. Regulation is achieved by a reversible dissociation process, in which the V_1 motor decouples from the V_O motor (Fig. 1). Subsequently, both motors are halted, silencing ATPase activity and proton flow [2].

The V_1 motor contains eight different subunits: *A*, *B*, *D*, *F*, *E*, *G*, *H* and *C* (Fig. 1). Subunits *A* and *B* form a trimer of heterodimers, with each dimer containing a catalytic site. The site binds ATP sequentially and hydrolyzes it to ADP, inducing conformational changes within the dimers. This mechanical movement is converted into rotation of a central rotor, comprised of subunits *D* and *F* [3]. Subunit *D* adopts a long crank-like shape that is pushed and rotated by the *AB* dimers, whereas subunit *F* is globular and is believed to be involved in regulating the complex [4]. Subunits *E* and *G* form the peripheral stalks, which attach the V_1 and V_O motors via long heterodimeric coiled coils [5]. Finally, subunits *H* and *C* link pairs of peripheral stalks forming a "collar" [3]. However, subunit *C* appears to dissociate from the complex during deactivation and subunit *H* has been suggested to inhibit ATPase activity in the dissociated V_1 complex [2,6].

In this issue of the *Journal of Molecular Biology*, Muench *et al.* present three-dimensional electron microscopy reconstructions of the deactivated V_1 from *Manduca sexta* to ~20 Å resolution, which contain sufficient detail to identify the "empty" state *AB* dimer. These reconstructions represent the most detailed view of the deactivated complex and allow for direct comparison with the intact, active enzyme [3]. Moreover, two reconstructions are presented of two complexes in different states: a fully deactivated state composed of the V_1 motor alone and a partially active state that also contains subunit *C*.

Multiple crystal structures of the peripheral stalks from related rotary ATPases have shown inherent flexibility in the coiled-coil domain of the stalk with the observation of multiple conformations [5,7,8]. Interestingly, this flection is always observed in a radial direction, perpendicular to that of rotation [9]. The reconstructions obtained by Muench *et al.* show all three peripheral stalks appearing as unsupported filaments, showing little differences between the deactivated and intact complexes. Although the stalks are most likely to have been observed in their lowest energy state, the fact that they were able to be resolved does suggest some inherent stiffness within the stalks that may be important for elastic power transmission between the motors. However, the lack of differences between peripheral stalk conformations in the deactivated V_1 motor and the intact V-ATPase shows that the peripheral stalks are unlikely to undergo bending upon subunit *C* binding that had been previously suggested [8].

By comparing the V_1 motor with and without subunit *C* present, Muench *et al.* have provided evidence to support the hypothesis that subunit *H* inhibits free V_1 by linking the rotary and stator domains [6]. Density, believed to correspond to subunit *H*, can be seen to link one of the peripheral stalks to the central rotor, which previous cross-linking data suggest could be subunit *F* [4]. This link could prevent rotation of the rotor and silence ATPase activity, just like an

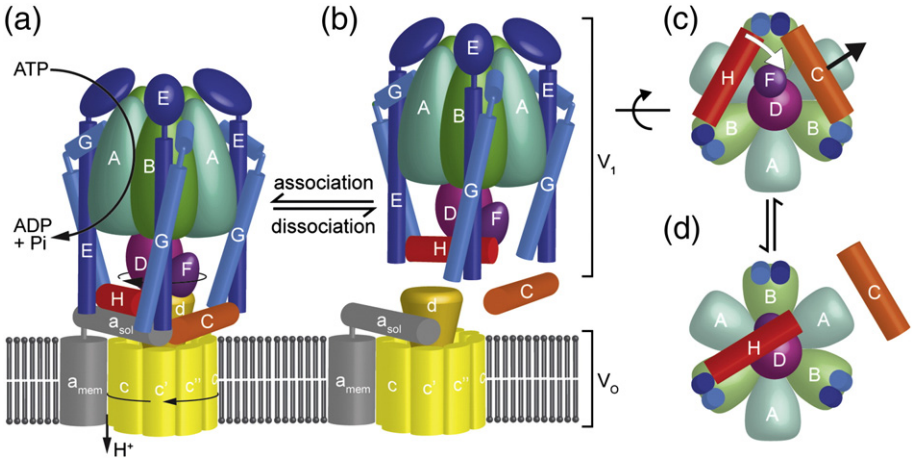

Fig. 1. The V-ATPase. Schematic diagrams of the V-ATPase with suggested mechanism of dissociation. (a) The intact V-ATPase. Arrows show ATP hydrolysis, direction of rotation and proton flow. Catalytic dimers are shown in shades of green; the central rotor, in shades of purple; the peripheral stalks, in shades of blue; the collar, in shades of red; and V_O, in gray and shades of yellow. (b) Dissociated V-ATPase with the V_1 and V_O motors labeled. (c) V_1 motor viewed from underneath. Arrows show suggested movement of subunits *C* and *σ*, black and white arrows, respectively. (d) The deactivated V_1 motor.

emergency brake. It is suggested that signals, most likely phosphorylation, acting upon subunit *C* could cause the subunit to detach from the peripheral stalks and hence the "collar" (Fig. 1c). This would lead to the weakening of the interaction between the V_O subunit *a* and the V_1 subunit *H*, facilitating dissociation of the complex. Subunit *H* would then be free to swing around to contact the central stalk and bind to subunit *F*, thereby preventing rotation and locking the motor.

In summary, Muench *et al.* have presented high-quality, three-dimensional electron microscopy reconstructions of the deactivated V_1-ATPase. Their data provide evidence for the biological process by which the V-ATPase is regulated to stop wasteful ATP hydrolysis. This can be thought of as a "fail-safe" mechanism, which would prevent the rotation of the motor under low load.

What is still unclear is the mechanism by which proton translocation is silenced in the V_O motor after it becomes dissociated from V_1. Future structural studies of the deactivated V_O motor may reveal an analogous mechanism, with the soluble domain of subunit *a* (a_{sol}) binding to subunit *d*. Ion-mobility mass spectrometry of the "$_O$" motor from the related A/V-ATPase from *Thermus thermophilus* has already shown the existence of nucleotide-dependent conformational heterogeneity in the transmembrane motor [10]. Analysis of these spectra indicated that the domain corresponding to a_{sol} was remarkably flexible, with its orientation being predicted to vary between 90° and 135° to the normal of the membrane plane. Thus, the V_O component of the deactivated complex will no doubt be a target of future structural studies.

References

[1] Stewart AG, Sobti M, Harvey RP, Stock D. Rotary ATPases: models, machine elements and technical specifications. Bioarchitecture 2013;3:2–12.
[2] Kane PM. Disassembly and reassembly of the yeast vacuolar H(+)-ATPase *in vivo*. J Biol Chem 1995;270:17025–32.
[3] Muench SP, Huss M, Song CF, Phillips C, Wieczorek H, Trinick J, et al. Cryo-electron microscopy of the vacuolar ATPase motor reveals its mechanical and regulatory complexity. J Mol Biol 2009;386:989–99.
[4] Jefferies KC, Forgac M. Subunit H of the vacuolar (H+) ATPase inhibits ATP hydrolysis by the free V1 domain by interaction with the rotary subunit F. J Biol Chem 2008;283:4512–9.
[5] Lee LK, Stewart AG, Donohoe M, Bernal RA, Stock D. The structure of the peripheral stalk of *Thermus thermophilus* H^+–ATPase/synthase. Nat Struct Mol Biol 2010;17:373–8.
[6] Parra KJ, Keenan KL, Kane PM. The H subunit (Vma13p) of the yeast V-ATPase inhibits the ATPase activity of cytosolic V1 complexes. J Biol Chem 2000;275:21761–7.
[7] Stewart A, Lee L, Donohoe M, Chaston J, Stock D. The dynamic stator stalk of rotary ATPases. Nat Commun 2012;3:687.
[8] Oot RA, Huang LS, Berry EA, Wilkens S. Crystal structure of the yeast vacuolar ATPase heterotrimeric EGC(head) peripheral stalk complex. Structure 2012;20:1881–92.
[9] Stewart AG, Stock D. Priming a molecular motor for disassembly. Structure 2012;20:1799–800.
[10] Zhou M, Morgner N, Barrera NP, Politis A, Isaacson SC, Matak-Vinkovic D, et al. Mass spectrometry of intact V-type ATPases reveals bound lipids and the effects of nucleotide binding. Science 2011;334:380–5.