Flexibility within the rotor and stator structures of the vacuolar ATPase

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Introduction

The vacuolar H^+-ATPase (V-ATPase) is a large transmembrane ATP driven proton pump which is essential in eukaryotic cells. Proton pumping is achieved through the coupling of a 10 stroke proton pump (V_o) and 3 stroke ATP driven motor ((V_1)(Figure 1 A)). The remarkable efficiency of this molecular motor is hypothesised to be due in part to the intrinsic flexibility in the stator connections and central rotor axle which links the two motors.

Results

In collaboration with the group of Dr Paci molecular dynamic simulations have been carried out on the previously determined 3D reconstruction of the *Manduca sexta* V-ATPase. These simulations revealed two modes of flexing. The first is flexing of V_1 relative to V_o to a maximum angle of 20° in the longitudinal direction (Figure 1C). The second mode is a twisting of V_1 relative to V_o about the equatorial region. In order to see if this predicted flexing could be directly visualised, electron microscopy was used to capture a range of conformational states in both the Yeast and Manduca V-ATPase using negative staining and cryo-EM approaches. Single particle processing has revealed a range of conformations whereby the V_1 domain can be seen to flex relative to V_o to a maximum of 30° in agreement with the molecular dynamic simulations (Figure 1B). This has provided direct evidence of the flexibility within the V-ATPase and by implication the rotary ATPase family which has significant implications in understanding their high energetic efficiencies.

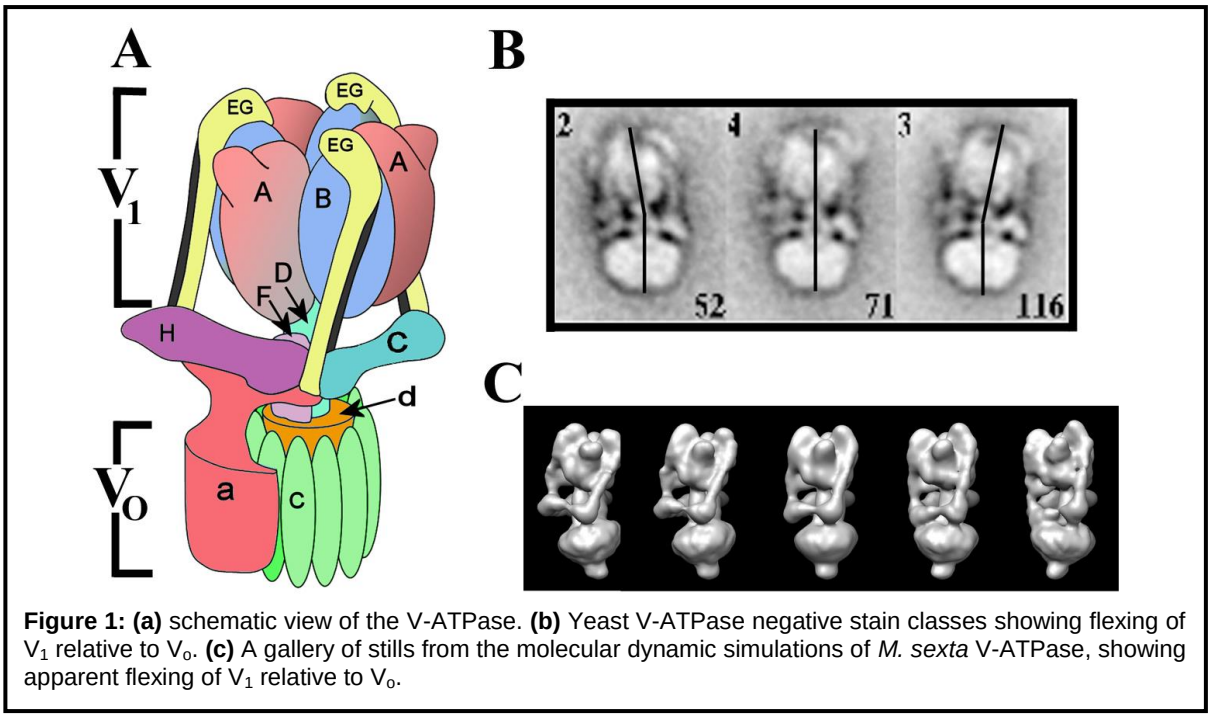

Figure 1: (a) schematic view of the V-ATPase. **(b)** Yeast V-ATPase negative stain classes showing flexing of V_1 relative to V_o. **(c)** A gallery of stills from the molecular dynamic simulations of *M. sexta* V-ATPase, showing apparent flexing of V_1 relative to V_o.

In addition to capturing the flexibility within the V-ATPase the group has also used single particle electron microscopy to study the structure of the isolated V_1 domain which is unable to turnover ATP. This structure has given us insights into the structural changes that bring about dissociation allowing us to propose a mechanism for V_1 dissociation. Moreover, the apparent rigidity of the stators within the isolated V_1 domain has implications for the elastic coupling model.

The mis-localisation of the V-ATPase plays a role in a number of disease states, such as Alzheimer's and cutis laxis disease. This localisation is thought to be driven through the glycosylation of several V-ATPase subunits, in particular the membrane bound *a* and *e* subunits. Electron microscopy and biochemical studies have shown that significant glycosylation of the V-ATPase is found at the base of V_o.

This work has been done in collaboration with Prof Wieczorek's group who provide us with *Manduca sexta* V-ATPase and some biochemical analysis. The yeast data was collected and processed by Chun Feng Song.

Publications

Fomovska, A., Huang, Q., El Bissati, K., Mui, E. , Witola, W., Cheng, G., Zhou, Y., Sommerville, C., Roberts, C., Bettis, S., Prigge, S., Afanador, G., Hickman, M., Lee, P., Leed, S., Auschwitz, J., Pieroni, M., Stec, J., Muench, S., Rice, D., Kozikowski, A. & McLeod, R. (2012) Novel n-benzoyl-2-hydroxybenzamide disrupts unique parasite secretory pathway. *Antimicrob. Agents. Chemother*. **56:** 2666-2682.

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