P029 Stator subunit organisation in the V-ATPase rotary molecular motor

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The vacuolar H^+-ATPases are membrane proteins that use the free energy of ATP hydrolysis to pump protons, acidifying the lumen of endomembrane compartments or extruding acid from the plasma membrane of some cells. Domain organisation of the V-ATPase resembles that of its simpler cousin the F_1F_0-ATPase, comprising a proton-translocating membrane domain V_0 and ATP hydrolysing domain V_1. Although the eukaryotic V-ATPase is more complex than the F-ATPase, integrating the function of as many as 14 different species of polypeptide, the two enzymes share a common rotary mechanism with essentially only two motor components- a rotor function that includes the membrane subunit-c involved in proton pumping, and a stator function that includes the ATP hydrolysing sites. In F-ATPase, electron microscopy has revealed only two stalk structures linking the soluble and membrane domains – a central stalk component of the rotor, and a second peripheral stalk that is part of the stator. However, EM analysis of the V-ATPase shows as many as four inter-domain stalk structures, complicating our understanding of the rotary mechanism in this ATPase. In our current work we have used electron microscopy to examine the structure and organisation of these stalks, and site-directed cross-linking to identify subunit contacts within them. As a result, we are able to propose a model for subunit organisation within these stalk structures.