

P028 Reversible dissociation of V-ATPases: fact or artefact?
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Primary active proton transport by eukaryotic V-ATPases is regulated via the reversible disassembly of the V_1V_O holoenzyme into its peripheral, catalytic V_1 complex and its membrane bound, proton translocating V_O complex. This nutrient dependent phenomenon had been first detected in the midgut epithelium of non-feeding, moulting tobacco hornworms (*Manduca sexta*), and in glucose deficient yeast cells (*Saccharomyces cerevisiae*). Immunocytochemical investigation of non-feeding starving instead of moulting tobacco hornworms revealed that subunits of the V_1 complex are, as expected, found in the cytoplasm, but that an astonishingly high amount appeared to be membrane bound. To clarify this surprising finding we investigated the reversible disassembly under approximately physiological conditions in living yeast cells, taking advantage of the existence of yeast strains with V-ATPase subunits fused to green fluorescent protein. By using yeast cells with fluorescent subunits of the V-ATPase, we found that only the V_1 subunit C is released after displacement of extracellular glucose with galactose, whereas the other V_1 subunits remain at or near the membrane. Neither disassembly nor reassembly of subunit C depends on the actin cytoskeleton, whereas disassembly but not reassembly depends on functional microtubules. Results from overlay blots and pull-down assays support the assumption that subunit C interacts directly with microtubules without involvement of linker proteins.