

**THE H⁺-PUMPING V-ATPASE OF HIGHER PLANTS: A VERSATILE
"ECO-ENZYME" IN RESPONSE TO ENVIRONMENTAL STRESS**

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The V-ATPase at the tonoplast of higher plants establishes and maintains an electrochemical proton-gradient across this membrane, which is the driving force of a wealth of transport processes of electrically charged and neutral solutes via uni-, sym- and anti-port carriers and ion-channels. Thus, this membrane enzyme has essential house-keeping functions in the life of plants. It is also involved in special mechanisms, such as turgor-dependent processes like extension growth, and movements like those of stomatal guard cells and pulvini. The essential life functions of the V-ATPase imply, that under environmental stress conditions it may need to undergo adaptive changes. This may involve a wealth of different environmental cues. Most well studied are responses to temperature (chilling; reviewed in [1]), mineral nutrition, salinity, oxidative stress (reactive oxygen species, ROS) and mode of photosynthesis (shifts between C₃-photosynthesis and crassulacean acid metabolism, CAM). We have investigated responses to NO₃⁻-nutrition, NaCl-salinity, ROS and C₃-CAM transitions, which are summarized here.

The V-ATPase is a complex multi-subunit enzyme. At the protein level the best known V-ATPase of higher plants is that of tobacco tonoplasts ([2]; see Figure 1). It has a head-structure consisting of the A (catalytic)- and B-subunits (evidence from MALDI-analysis; 3 copies each), a stalk consisting of the C, D, E, F and G subunits (evidence from amino-acid sequencing and MALDI-analysis). Head and stalk build up the V₁-domain. Its V_o-domain constitutes a membrane integral structure (visible in freeze-fracture replicas as spherical particles) with a c and a d subunit (evidence from amino-acid sequencing and MALDI-analysis). Subunits known from V-ATPases in other materials, such as the H-subunit of the stalk and the c'-, c''- and a-subunits of the membrane integral V_o-domain remain putative.

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In response to NO_3^- , NaCl, ROS and C_3/CAM -transitions we found alterations in the subunit fine-structure, amount, activity and transcription of the V-ATPase. Hence, we named it an "eco-enzyme" [3].

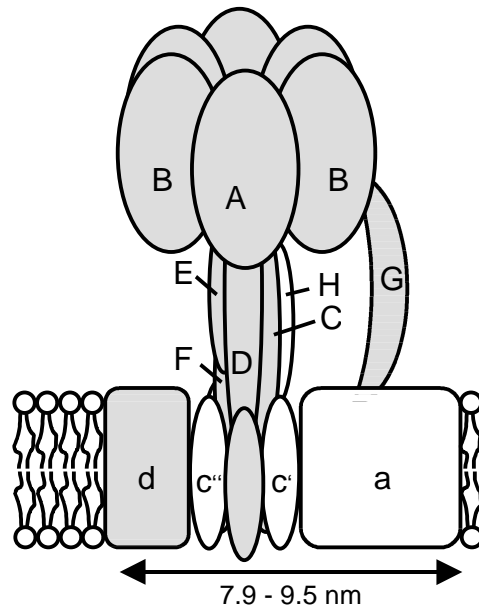


Fig. 1. Structural model of the *Nicotiana tabacum* L. V-ATPase based on the subunit composition of the yeast V-ATPase [4]. Subunits which have been identified in tobacco by amino acid sequencing and/or MALDI analysis are given in grey (for details see text).

Nitrate nutrition of tobacco

Plants of *Nicotiana tabacum* L. cv. Samsun were grown at limiting (2 mM NO_3^-) and luxury (40 mM NO_3^-) nitrate concentrations and also transferred from 2 mM to 40 mM NO_3^- for 48 h [5]. At 2 mM NO_3^- as compared to 40 mM NO_3^- the diameter of the V_o intramembrane-particles (IMPs) shifted to larger values. Responses of subunit c were different for two different antisera against the V-ATPase holo-enzyme raised in two different rabbits. One of the two antisera stained subunit c of the plants with the three different NO_3^- -regimes to different extents. The coupling ratio of H^+ -transport and ATP-hydrolysis measured simultaneously in a test of Palmgren [6] was lowest at 40 mM NO_3^- , higher at 2 mM NO_3^- at highest upon a transfer from 2 to 40 mM NO_3^- . We conclude that structural changes at the V_o -level, i.e. perhaps exprimation of different subunit- c isoforms, affect ATP hydrolysis and H^+ -transport in different ways. The

increased coupling ratios then reflect increased demands on H^+ -transport into the vacuole (NO_3^- -surplus) as well as neutralization of OH^- generated during NO_3^- -reduction via synthesis of malic acid and transport of malate together with K^+ into the vacuoles.

NaCl-salinity in *Citrus sinensis* (L.) Osbeck

The major involvement of the V-ATPase in salinity stress-responses is energization of the vacuolar Na^+/H^+ -antiporter (for review see [7]) for Na^+ -sequestration in the vacuole. Some salinity responses of kinetics and activities of the V-ATPase of various materials were reported in the literature, while in other cases V-ATPase activities in salt-sensitive and salt-resistant species were found to be rather similar [8]. Some root-stocks of *Citrus* cultivars are tolerant of medium salinity stress. In *Citrus sinensis* we found that salinity *per se* in this obligate C_3 plant leads to appearance of a proteolytic breakdown product of subunit A of the V-ATPase head, which still showed ATP-hydrolysis activity [9], i.e. salinity affects the V-ATPase at the structural level.

C_3 -CAM shift in *Kalanchoë blossfeldiana* Poellnitz cv. Tom Thumb

Under short-day photoperiod-conditions *Kalanchoë blossfeldiana* shifts its metabolism from C_3 photosynthesis to CAM. This leads to a higher expression of the V-ATPase (subunits A, B, C, D, c) [10]. Thus CAM induction *per se* leads to increased amounts of the V-ATPase. An essential feature of the CAM cycle of CO_2 -assimilation is a massive nocturnal accumulation of organic acid, mainly malic acid, driven by the V-ATPase and, hence, requiring such a change in V-ATPase amount.

NaCl-salinity and C_3 -CAM shift in *Mesembryanthemum crystallinum* L.

Most work has been performed on the annual halophyte *Mesembryanthemum crystallinum* L. Here C_3 -CAM shift is elicited by NaCl-salinity. Hence, it is not easy to study either cue *per se*. With salinity and CAM induction the following changes of the V-ATPase were observed.

1. The amount and activity increased [11-13].
2. The coupling ratio of H^+ -transport and ATP hydrolysis decreased [14]. We may assume that this is due to the V-ATPase having to pump protons against a steeper electrochemical gradient in CAM with its strong nocturnal acid accumulation. This decline in coupling ratio amplifies the need for a larger amount (see (1)) of the enzyme with the special requirements of CAM given.
3. The IMP-diameter of V_o increases [15,16]. This is accompanied by an increase in the levels of subunit-c protein-amounts and the amounts of mRNA at dawn [17]. Thus, the change of coupling ratio may not only have thermodynamic reasons (see (2)) but also structural reasons as in tobacco.

4. Two new subunits, D_i and E_i, appear in the stalk. D_i is a proteolytic breakdown product of subunit-B due to the function of a specific protease *in vivo* [18,19].
5. Cleavage of subunit-B is not only mediated by a protease but also by ROS [19]. This may reflect (i) the fact that salinity often causes oxidative stress in plants, and it adds to the observation of subunit-A proteolytic breakdown in *Citrus*; (ii) the special oxidative stress in phase III of CAM when CO₂ regenerated from nocturnally stored malate is assimilated with strong concomitant photosynthetic O₂-evolution behind closed stomata [20].
6. The V-ATPase head in electron-micrographs of negatively stained tonoplast vesicle-preparations with image enhancement shows both hexameric and pentameric structures [21]. Thus, not always three copies of each subunit, A and B, are present. This must represent assembly/disassembly/proteolytic-attack dynamics. It is not known whether the pentamer misses a subunit A or B. It is noteworthy in this respect that there is proteolytic breakdown of subunit-A under salinity in *Citrus* but of subunit-B in *M. crystallinum*. In the latter mRNA levels of subunit-B at dawn are markedly increased with NaCl-salinity and C₃-CAM shifts but those of subunit-A to a much smaller extent [17].

In conclusion, the versatile structural changes of the V-ATPase of *M. crystallinum* in all domains, the responses of transcription (mRNA-levels) and translation (protein-levels) as well as activities (coupling ratios) demonstrate that the V-ATPase is an eco-enzyme with considerable plasticity. The other examples of *N. tabacum*, *C. sinensis* and *K. blossfeldiana* add to this contention.

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