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Minireview

Structure, function and regulation of plasma membrane H^+-ATPase

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Most antigenic determinants of yeast ATPase are located within its N-terminal part. Amino acids 24–56, required for insertion at the plasma membrane, are highly accessible. The C-terminus behaves as a modulable auto-inhibitory domain in both yeast and plant ATPases. The expression of functional plant enzyme in yeast allows its mutational analysis. Plant tissues involved in active transport, such as the stomata guard cells, phloem, root epidermis and endodermis, are enriched in ATPase. One isoform is phloem-specific. The fact that auxin induces the synthesis of ATPase in corn coleoptiles provides molecular support to the 'Acid growth' theory.

ATPase; Epitope mapping; Inhibitory domain; Auxin; Immunolocalization

1. INTRODUCTION

The study of plasma membrane H^+-ATPase has moved in the last years from the fields of physiology and biochemistry [1,2] to molecular, genetic and immunological approaches which have provided novel clues to the structure, function and regulation of the proton pump [3,4].

Some developments in which my laboratory has directly participated during the last years, will be described below. Other recent findings which deserve attention are briefly listed. The cloning of *Candida albicans* ATPase [5] may facilitate the rational design of badly needed antifungal drugs. In *Saccharomyces cerevisiae*, the analysis of intragenic revertants has provided evidence for coupling between transmembrane and cytoplasmic domains [6]. A new strategy for directed mutagenesis based on expression of mutant ATPases in yeast secretory vesicles has been developed [7]. The participation of the ATPase in the heat-shock response has been demonstrated [8]. ATPase mutants resistant to Dio-9 have been characterized at the molecular and biochemical levels [9,10].

2. EPITOPE MAPPING AND ACCESSIBILITY OF YEAST ATPase

The amino- and carboxyl-termini of *Neurospora* [11,12] and *Saccharomyces* [13,14] ATPases are cytoplasmically located. This is consistent with the interpretation that this polytopic membrane protein spans the membrane an even number of times (6, 8 or 10, according to different models).

A cytoplasmic region within the N-terminal part of yeast ATPase (at amino acid positions 24–105) contains most of the antigenic determinants [14]. It is divided into six epitopes (Fig. 1A). Epitopes A, C and G, close to the membrane surface, are only accessible to monoclonal antibodies after structural perturbation with detergents or organic solvents. On the other hand, the most terminal epitopes D, E and H are highly accessible in unperturbed membrane preparations. They encompass a very acidic stretch of amino acids predicted to fold as an α-helix (Fig. 1A). This region of the ATPase is not conserved in non-fungal ATPases, and it has been shown by deletion analysis to be essential for the appearance of the enzyme in the plasma membrane [15]. Tryptic cleavage studies with the *Neurospora* ATPase also demonstrated an essential role for this part of the enzyme [16]. It is probably required for the correct folding and targetting of the ATPase. It would be interesting to find out what kind of proteins interact with such a highly exposed and essential part of the enzyme.

3. AUTO-INHIBITORY DOMAIN AT THE C-TERMINUS OF YEAST AND PLANT ATPases

The yeast ATPase is activated in vivo by some signal triggered by glucose metabolism [17]. Removal of the last 11 amino acids (Fig. 1B) produces an enzyme in glucose-starved cells with the properties of wild-type ATPase activated by glucose [15]. Arg^{909} and Thr^{912} within this terminal region are important for regulation, suggesting a phosphorylation mechanism [18]. Actually,

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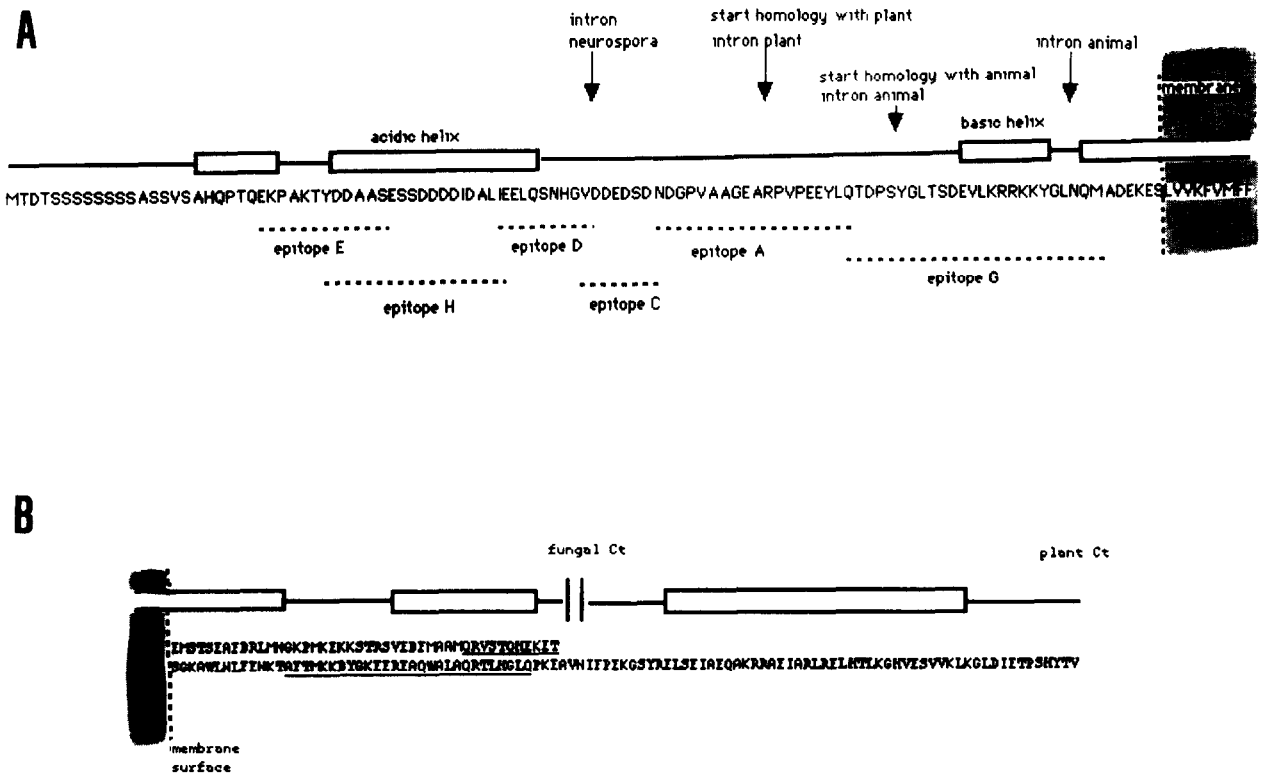

Fig. 1. Structural features of amino- and carboxyl-termini of plasma membrane H^+-ATPase. (A) Sequence of the *Saccharomyces* ATPase (*PMA1*) up to the first predicted transmembrane stretch. Mapped epitopes are indicated below the sequence and predicted α-helices (rectangles), intron positions and begining of homology with other ATPases are shown above the sequence. (B) Aligned sequences of the carboxyl-termini of *Saccharomyces* ATPase (*PMA1*) (upper sequence) and *Arabidopsis* ATPase (*AHA2*) (lower sequence) after the last predicted hydrophobic stretch. The inhibitory domains of both enzymes are underlined and the predicted α-helices (rectangles) are indicated above.

glucose activation of the ATPase is acompanied by the appearance of a novel phosphopeptide in thermolysin digests of the enzyme [19].

A double mutation at the carboxyl-terminus (Ser^{911}→Ala; Thr^{912}→Ala) greatly reduces the activation of the ATPase by glucose [18]. On the other hand, a mutation (Ala^{547}→Val) within the ATP binding site locks the enzyme in its high activity state, with independence from glucose metabolism [20]. When combined in the same gene, the active site mutation suppresses the effect of the carboxyl-terminus mutations [18]. Therefore, it has been proposed that the last 11 amino acids of the ATPase form part of an auto-inhibitory domain interacting with the active site and that phosphorylation of the ATPase triggered by glucose metabolism decrease this interaction [18]. Some detergents, such as lysophosphatidic acid, activate the ATPase [21], and this could be explained by a displacement of the carboxyl-terminus, which, in the presence of detergents, is more accessible to antibodies [13].

This mechanism of regulation seems also to apply to the plant ATPase, where removal of the carboxyl-terminus at either the protein [22] or gene [23] level activates the enzyme. In addition, a peptide including the region of homology to the yeast inhibitory domain (Fig. 1B) inhibits the enzyme activated by proteolysis but not the intact ATPase [22]. Detergents such as lysolecithin activate intact ATPase but not the enzyme deleted from the carboxyl-terminus [22,23]. Again, they could displace the carboxyl-terminus. The physiological factors regulating the activity of plant ATPase are not so well characterized as in the case of the yeast enzyme. Activation by the phytotoxin, fusicoccin, has recently been reconstituted with partially purified preparations of ATPase and fusicoccin receptor [24,25]. Light effects on the enzyme seem to be mediated by calcium-calmodulin protein kinases [26] and phosphoinositide changes [27].

4. PARTIAL CONSERVATION OF INTRON POSITIONS IN EUKARYOTIC ATPases

The recent sequencing of a genomic clone of *Nicotiana* ATPase [28] has complemented the intron positions determined for *Arabidopsis* ATPase [29,30]. The five extra introns of the *Nicotiana* gene are conserved in animal ATPase genes [28,29]. The relationship between exons and the predicted secondary and transmembrane structure of different ATPases with phosphorylated intermediate support a correspondence between exons and structural modules [31].

5. TISSUE DISTRIBUTION OF PLANT ATPase

Immunocytolocalization of plant ATPase with specific antibodies [32–34] has revealed that the enzyme is enriched in tissues specially involved in active transport, such as the stomata guard cells (Fig. 2B), phloem, xylem parenchyma, root epidermis, endodermis and pericycle. One isoform of *Arabidopsis* ATPase has been shown to be phloem-specific [35], and it is likely that differential tissue distribution provides the rationale for the existence of different isoforms [28–30].

6. FUNCTIONAL EXPRESSION OF PLANT ATPase IN YEAST

The *Arabidopsis* ATPase can be expressed in functional form and large amounts in yeast. The complete enzyme accumulates at the endoplasmic reticulum [36], a convenient location to achieve easy separation from the yeast ATPase which is in the plasma membrane (Fig. 2A). On the other hand, a carboxyl-terminal deletion of *Arabidopsis* ATPase leads to targeting to the yeast plasma membrane and can support yeast growth in mutants without functional yeast ATPase [23]. This opens the way for a detailed mutational analysis of the plant enzyme. Both the enzymological and transport properties of isolated isoforms and mutant plant ATPases, and the active tranport properties of whole yeast cells expressing them, can be measured.

7. AUXIN INDUCES THE SYNTHESIS OF ATPase IN CORN COLEOPTILES

The acid growth theory of auxin-induced cell elongation [37] missed a molecular link between the hormone and the proton pump. The recent demonstration that auxin induces the synthesis of an special pool of ATPase in corn coleoptiles [38] may provide this mechanism. The working hypothesis is that there is an isoform of plant ATPase responsive to auxin and mediating growth responses.

8. THE MOLECULAR PHYSIOLOGY OF THE PROTON PUMP

The significance of plasma membrane H^+-ATPase for cell and tissue physiology can now be determined with the rigor of molecular biology. In yeast this analysis is quite advanced, with mutant studies demonstrating the role of the ATPase in proton pumping and in pH homeostasis under in vivo conditions [3]. In plants this mutational analysis has not yet started. We need plants with alterations in ATPase to ascertain the participation of the enzyme in all its proposed physiological roles [3]. Transgenic plants with tissue-specific promoters expressing extra ATPases or isoform-specific antisense RNAs could provide the required tools.

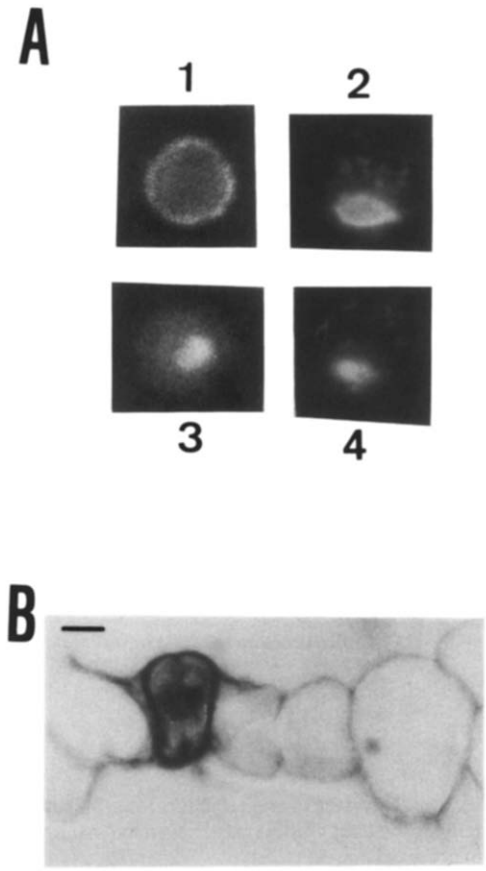

Fig. 2. Immunocytolocalization of plasma membrane H^+-ATPase. (A) Fluorescence images of a control yeast cell (1 and 3) and of a yeast cell expressing *Arabidopsis* ATPase (2 and 4). Cells were DAPI-stained for nuclei (3 and 4) or immunodecorated with antibody against yeast ATPase (1) or *Arabidopsis* ATPase (2) and second antibody coupled to fluorescein. Note localization of yeast ATPase at cell surface and *Arabidopsis* ATPase at the endoplasmic reticulum surrounding the nucleus. (B) Corn leave epidermis immunodecorated with antibody against corn ATPase and second antibody coupled to alkaline phosphatase. Note enrichement of ATPase in stomata guard cells. Bar = 5 μm.

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