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Regulation of the plant plasma membrane H$^+$-ATPase by its C-terminal domain: what do we know for sure?

Corinna Speth, Nina Jaspert, Caroline Marcon, Claudia Oecking*

Center for Plant Molecular Biology – Plant Physiology, University of Tübingen, Auf der Morgenstelle 5, 72076 Tübingen, Germany

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ABSTRACT

The plant plasma membrane H$^+$-ATPase is kept at a low activity level by its C-terminal domain, the inhibitory function of which is thought to be mediated by two regions (region I and II) interacting with cytoplasmic domains essential for the catalytic cycle. The activity of the enzyme is well known to be regulated by 14-3-3 proteins, the association of which requires phosphorylation of the penultimate H$^+$-ATPase residue, but can be abolished by phosphorylation of residues close-by. The current knowledge about H$^+$-ATPase regulation is briefly summed up here, combined with data that query some of the above statements. Expression of various C-terminal deletion constructs of PMA2, a H$^+$-ATPase isoform from *Nicotiana plumbaginifolia*, in yeast indicates that three regions, which do not correspond to regions I or II, contribute to autoinhibition. Their individual and combined action can be abolished by (mimicking) phosphorylation of three threonine residues located within or close to these regions. With respect to the wild-type PMA2, mimicking phosphorylation of two of these residues increases enzyme activity. However, constitutive activation of wild-type PMA2 requires 14-3-3 association. Altogether, the data suggest that regulation of the plant H$^+$-ATPase occurs in progressive steps, mediated by several protein kinases and phosphatases, thus allowing gradual as well as fine-tuned adjustment of its activity. Moreover, mating-based split ubiquitin assays indicate a complex interplay between the C-terminal domain and the rest of the enzyme. Notably, their tight contact does not seem to be the cause of the inactive state of the enzyme.

Introduction

The plasma membrane H$^+$-ATPases of plants and fungi are primary active pumps that couple ATP hydrolysis to proton transport out of the cell. The established pH and potential gradients across the plasma membrane provide the driving force for secondary transport of metabolites. In addition to its fundamental role in nutrient uptake, the plant enzyme plays a major role in processes essential for normal growth, such as stomatal movement, cell elongation, pH homeostasis, salt tolerance (reviewed in (Palmgren, 2001; Duby and Boutry, 2009)) and also pathogen attack (Liu et al., 2009). Several of these require balanced regulation, suggesting that multiple regulatory pathways may converge in order to fine-tune H$^+$-ATPase activity. P-type H$^+$-ATPases are characterized by C-terminal domains serving as autoinhibitors (Baekgaard et al., 2005). The mechanism of pump activation, however, seems to differ significantly between plants and fungi, since their respective autoinhibitory domains do not share any sequence homology and, moreover, are of different length. With regard to the latter, the plant domain contains twice as many amino acids (ca. 100) as compared to the fungal domain, suggesting a more complex regulation of the plant enzyme.

On the basis of mutagenesis experiments, as well as systematic alanine scanning, two regions (regions I and II, Fig. 1) have been identified which seem to be important for the autoinhibitory function of the C-terminal domain of the plant enzyme, more precisely the *Arabidopsis thaliana* isoform AHA2 (Baunsgaard et al., 1996; Axelsen et al., 1999) and PMA2 from *Nicotiana plumbaginifolia* (Morsomme et al., 1996, 1998). Since mutations within these regions abolish the inhibitory effect of the C-terminus, they have been assumed to interact intramolecularly with the rest of the pump, which, however, is still to be verified.

Moreover, the constraint exerted by the autoinhibitor is released upon binding of 14-3-3 dimers (Oecking et al., 1997; Piotrowski et al., 1998; Fuglsang et al., 1999; Svennelid et al., 1999; Maudoux et al., 2000; Jaspert and Oecking, 2002), which are well known as discrete phosphoserine/threonine-binding modules that operate by enforcing conformational changes, among others (MacKintosh, 2004). Interaction with 14-3-3s *(i)* requires phosphorylation of the penultimate H$^+$-ATPase residue (Fuglsang et al., 1999; Svennelid et al., 1999; Maudoux et al., 2000), a highly

* Corresponding author. Tel.: +49 7071 297 6679
E-mail address: claudia.oecking@zmbp.uni-tuebingen.de (C. Oecking).

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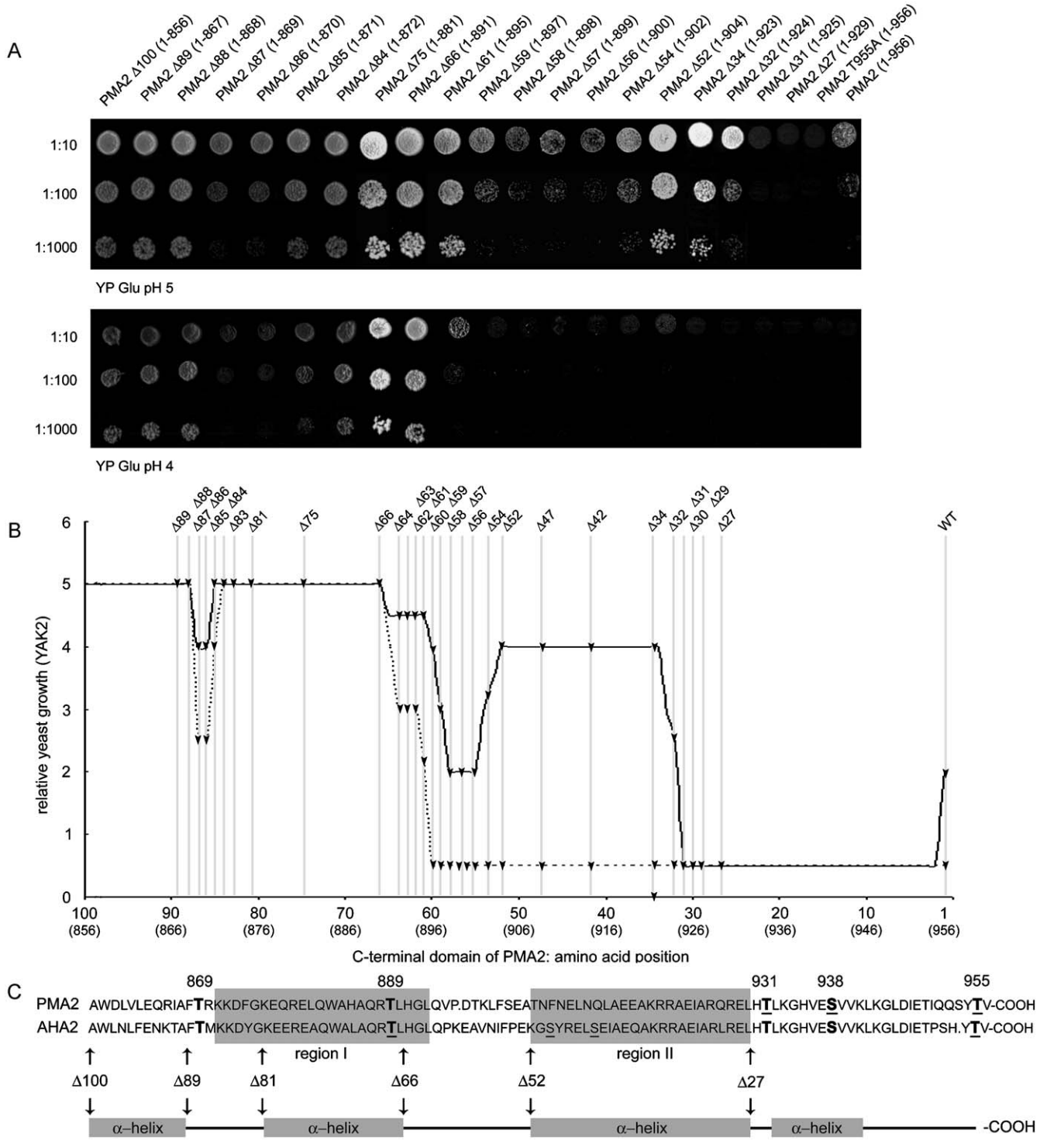

Fig. 1. (A) Growth of YAK2 transformed with wild-type and C-terminal deletion mutants of PMA2. Each strain was spotted at serial 10-fold dilution onto solid rich medium (pH 5.0 and pH 4.0, respectively), containing glucose as a sole carbon source. (B) Relative growth of YAK2 expressing the indicated PMA2 versions. The yeast growth was classified between 0 and 5, with category 0 indicating no and category 5 indicating strong growth. (C) Amino acid sequence of the C-terminal regulatory domains of PMA2 and AHA2. Region I and II are marked by grey background. Phosphorylated residues described in the literature are underlined and their homologs are shown in bold. The bars correspond to helices, that are either resolved by a crystal structure (downstream of Δ52; Ottmann et al., 2007) or predicted (secondary structure prediction according to PROF).

conserved threonine, *(ii)* involves approximately 50 C-terminal H^+-ATPase residues (Jelich-Ottmann et al., 2001; Fuglsang et al., 2003) (see Fig. 7) – altogether constituting the part that is absent in fungal pumps, and *(iii)* results in formation of a H^+-ATPase hexamer (Kanczewska et al., 2005; Ottmann et al., 2007). The crystal structure of 14-3-3, in complex with the entire binding motif, clearly shows that the C-terminal 27 residues are directly bound within the typical amphipathic groove of a 14-3-3 monomer, while the remaining amino acids, which correspond to region II (Fig. 1), protrude from the center of the 14-3-3 dimer (Ottmann et al., 2007) (see Fig. 7).