SESSION 5: INTRACELLULAR TRAFFIC

P053

CYTOTOXIC DRUG RESISTANCE IN YEAST AND ANIMAL CELLS BY FUNCTIONAL COMPLEMENTATION OF V-ATPASE WITH PLANT PROTON-TRANSLOCATING MEMBRANE PYROPHOSPHATASES

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The vacuolar ATPase (V-ATPase) is a complex primary proton pump essential for the generation of electrochemical gradients in endomembrane systems. Another structurally simpler ion pump that co-localizes with the V-ATPase in plants and many protists is the H+-translocating inorganic pyrophosphatase (H +-PPase), consisting of a single subunit. The expression of translational fusions of the N-terminus of the H +-PPase of the protist Trypanosoma cruzi with its vacuolar ortholog AVP1 of the plant Arabidopsis thaliana directed these chimeras preferentially to the inner membranes of S. cerevisiae, effectively relieving the phenotypes associated with deficiency in V-ATPase provided the cytosolic PPase is inactive. This phenotypic complementation was achieved whether the V-ATPase was specifically inhibited by cytotoxic agents (bafilomycin A1) as with the mutant strain Vma1, deficient in an essential catalytic subunit of the V-ATPase. Using fluorescent dyes, it was shown that acidification of the vacuole is restored and the endocytic pathway is normalized in vma- mutants expressing heterologous H +-PPases, which are localized mainly in the vacuolar membrane. Biochemical and immunochemical studies showed that these H +-PPases are properly processed. These results demonstrate the ability of the H +-PPase to generate by itself physiologically competent pH gradients. Moreover, they raise new ways of engineering cytotoxic drugs tolerance in fungal and animal cells, and outline an experimental system suitable for studying alternative functions for V-ATPase, other than the mere acidification of intracellular organelles.

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