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CHARACTERIZATION OF HUMAN ALPHA-MANNOSIDASE SECRETORY PATHWAY IN TOBACCO PLANTS

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Traffic of α-mannosidases to the lysosomal or vacuolar compartment follows alternative routes in different kingdoms; In animal cells, these hydrolases are transported to lysosomes by the mannose 6-phosphate pathway, in plants, vacuolar α-mannosidase is targeted to its final destination via the classic secretory pathway involving the ER-Golgi system, whereas in yeast vacuolar delivery of α-mannosidase can be reached by both cytoplasm to vacuole targeting (Cvt) and autophagy pathways. Recently, tobacco plants expressing a human lysosomal α-mannosidase (MAN2B1) have been obtained. In tobacco leaves, the recombinant enzyme was found to be N-glycosylated and localised in vacuolar compartments, even if the plant counterpart of the mannose 6-phosphate pathway is not known. We then tried to understand what kind of mechanism uses this human protein in the plant to reach the vacuole. To study the traffic of the precursor MAN2B1 polypeptide, transgenic leaf protoplasts were incubated in the presence of the fungal toxin brefeldin A (BFA) and then pulse-chase analysed. Protoplasts were homogenated and immunoprecipitated with anti-MAN2B1 antiserum. BFA negatively affects Golgi-mediated protein traffic and in presence of this toxin the traffic of proteins to the vacuole is inhibited. Conversely, the 110-kD MAN2B1 precursor is substantially not affected by the addition of BFA and this suggests that the traffic to the vacuole of the precursor MAN2B1 polypeptide is not dependent on Golgi-mediated delivery. To confirm the results of the pulse-chase analysis, an aliquot of the BFA-treated protoplasts was subjected to microscopy analyses. Immunolocalization of MAN2B1 indicated that the protein was mainly detectable as small and delimitated structures, which did not change after BFA addition, confirming the existence of a MAN2B1 route to vacuoles which does not pass through the Golgi apparatus. We are performing other analyses on transformed tobacco seeds to characterize the MAN2B1 route to vacuoles. Moreover, the MAN2B1 polypeptide does not contain known plant sorting signals that would direct it to the vacuole. Therefore, we are investigating which structural part of the protein acts as a vacuolar targeting signal.