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Poster Abstract – E.11

INVESTIGATING RECOMBINANT PROTEIN STABILITY IN THE CHLOROPLAST: THE ROLE OF DISULFIDE BONDS

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In the last two decades there have been growing research efforts on the production of foreign proteins in the chloroplast. The accumulation of a recombinant protein is often increased by chloroplast targeting, even if the wild-type protein is naturally a resident of the cytosol or other compartments. Nevertheless, the results can be unsatisfactory also using these strategies: most likely the chemical environments and repertoire of "folding helpers" of the plastids are not universally optimal and therefore are not suited to any protein.

We have recently produced a chimeric protein, zeolin, that contains domains of the maize prolamin gamma-zein fused with the complete sequence of bean phaseolin. Like gamma-zein, zeolin forms large insoluble polymers in the ER, termed protein bodies (PBs), and accumulates to very high levels in vegetative tissues of transgenic plants. Inter-chain disulfide bonds are a determinant for zeolin PB formation and ER retention. Thus, zeolin is a prototype to study plants as bioreactors for producing pharmaceuticals and other industrial proteins. Unfortunately, when we expressed zeolin in chloroplasts of transplastomic plants, it was largely fragmented and accumulated to unsatisfactory levels, indicating more pronounced proteolytic activity towards zeolin in this compartment than in the ER. Two possible explanations for zeolin poor accumulation in the chloroplast are: (i) zeolin may be highly unstable (very low half-life) because in its native conformation it is recognized as a substrate by chloroplast proteases; (ii) inefficient or partial formation of inter-chain disulfide bonds may cause zeolin misfolding and degradation by the chloroplast protein quality control system. Zeolin does not form PBs in chloroplast, nor do zeolin polypeptides seem to be efficiently linked by inter-chain disulfide bonds, which in the ER are formed by Cys residues of the gamma-zein portion. These results indicate abnormal folding of the recombinant protein, favouring the hypothesis of quality control degradation.

The present research aimed at identifying the defects that limit zeolin accumulation in the chloroplast. By pulse-chase experiments we determined that the half-life of intact zeolin in chloroplasts is around 8 h, indicating that its fragmentation is a slow post-translational process. We are producing transplastomic plants that express a mutated form of zeolin in which the cysteine residues have been replaced with serine residues (zeolinCys-). We previously determined that, when the transgene is inserted into the nucleus, zeolinCys- enters in the ER and is then secreted, indicating that it is unable to form PB but it is not recognized as a structurally defective polypeptide by the ER protein quality control. We want to verify if the absence of disulfide bonds stabilizes zeolin in the chloroplasts or makes it more unstable. Analysis of the transplastomic plants expressing zeolinCys- is in progress. The results will give us information both on the relevance of disulfide bond formation in the chloroplast for zeolin folding and stability and the possible differences between the protein quality control of the two compartments (chloroplast vs ER).