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PROGRESSIVE COALESCENCE OF 16KD GAMMA-ZEIN IN DEVELOPING SEEDS OF TRANSGENIC ARABIDOPSIS

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The polypeptides of seed storage proteins undergo different kinds of inter-chain interactions that are important for their synthesis, intracellular traffic and deposition. At the end of seed development and irrespectively to their final subcellular storage compartment - protein storage vacuoles (PSV) or protein bodies (PB) directly derived from the endoplasmic reticulum (ER) - storage proteins appear at the electron microscope (EM) as highly electron dense structures. The biogenesis of these structures are however quite variable, depending on the specific protein and the tissue of accumulation (endosperm or embryo). Unlike the two major classes of PSV storage proteins - 7S/11S globulins and the monomeric 2S albumins - which are easily solubilized in aqueous buffers, the major storage proteins of cereals - prolamins - form very large PBs in the ER by extensive inter-chain disulfide bonds that lead to protein insolubilization. Zeins, the prolamins of maize, can be divided into four classes: alpha- (more than 30 genes), gamma- (3 genes for polypeptides of 27, 50, and 16 kD), beta- and delta-zeins (both single genes). alpha- and delta-zeins constitute the core of the PB, whereas 27 and 50 gamma-zeins (27gz and 50gz) form the outer PB layer, in contact with the luminal face of the ER membrane, and 16 gamma-zein (16gz) is at the interface between the inner core and outer PB layer. 27gz, the single most abundant PB polypeptide forms homotypic, insoluble, electron dense PB also when ectopically expressed in vegetative tissues. 27gz and 50gz have orthologs in other Panicoideae, whereas 16gz has been found only in maize, where it originated with the relatively recent whole genome duplication. With respect to the 27gz protein, it has lost part of

the cysteines involved in interchain bonds and large portion of a Pro-rich repeated domain. Unlike 27gz, 16gz is unable to form PB when expressed ectopically in transgenic vegetative tissues, where instead it polymerizes into disordered electron dense threads that markedly enlarge the ER lumen. 16gz is partially soluble, but it becomes fully insoluble if co-expressed with 27gz, in agreement with the hypothesis that in a natural PB it helps to establish ordered interactions between the inner PB core and the outer layer. We have investigated whether the peculiar features of 16gz are also maintained when the protein is ectopically synthesized during seed development, when other types of storage proteins are synthesized as well, and where a development program is active to favor their optimal accumulation. We show that during seed development in transgenic Arabidopsis, 16gz progressively coalescences into insoluble PB. In transgenic mature seeds, 16gz and 27gz appear very similar at the EM. However, when treated with reducing agent, the latter are fully soluble whereas the former remain largely insoluble, strongly suggesting aggregation in a storage tissue if expressed in the absence of the other zein partners.