

A γ -ZEIN-NEF FUSION IS UNSTABLE IN TRANSGENIC TOBACCO LEAVES

M. BELLUCCI*, M. DE VIRGILIO**, F. DE MARCHIS*, S. ARCIONI*, E. BENVENUTO***, A. VITALE**

*) Istituto di Genetica Vegetale, CNR, Perugia

**) Istituto di Biologia e Biotecnologia Agraria, CNR, Milano

***) ENEA, UTS Biotecnologie, Sezione Genetica e Genomica Vegetale, Casaccia, Roma

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Plants are being investigated as a possibly safer and less expensive alternative to microorganisms, animal cell cultures or transgenic animals for the production of recombinant pharmaceuticals. In this respect, production of antibodies has proved to be qualitatively and quantitatively efficient, whereas recombinant antigen accumulation has often been unsatisfactory. We are trying to produce high amounts of recombinant Nef, a human immunodeficiency virus antigen, in transgenic tobacco by taking advantage of the high stability of proteins that form protein bodies within the endoplasmic reticulum of plant cells. Nef is a cytosolic protein that has been up to now recalcitrant to high accumulation in plants. We have previously shown that an N-terminal domain of the maize seed storage protein g-zein fused to the vacuolar plant protein phaseolin highly enhances the accumulation of the latter expressed in leaves of transgenic plants, most probably because the chimeric protein (termed zeolin) forms very stable protein bodies within the endoplasmic reticulum, thus avoiding degradation in vacuoles or other compartments of the secretory pathway. A similar γ -zein-Nef fusion was constructed and expressed in transgenic tobacco. In spite of the high accumulation of mRNA, γ -zein-Nef protein accumulates at low levels. Pulse-chase analysis indicates that the protein has a half-life of less than 2 hours and is degraded in a process that cannot be blocked by brefeldin A, an inhibitor of traffic along the secretory pathway. These results suggest that the fusion protein, unlike zeolin, may be recognized as a structurally defective protein by the endoplasmic reticulum quality control mechanisms. These results indicate that the ability of the zein domain to promote stable protein body formation is influenced by the target protein used to produce the fusion. The structural features that determine the different destinies of zeolin and γ -zein-Nef are under investigation. This can increase our knowledge on the regulation of protein quality control in plant cells and help in the planning of further, possibly more stable, constructs.

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