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Poster Abstract - A.04

TAIL-ANCHORS FROM TYPE IV MEMBRANE PROTEINS AS A TOOL TO BIND ANTIGENIC MOLECULES TO THE ER MEMBRANE

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Tail-anchored, membrane proteins, cytochrome b5, antigens, HIV

Transgenic plant as bioreactor has been used to produce recombinant proteins for medicinal purposes, including mammalian antibodies, blood substitutes and vaccines, because they represent a safe, effective, and inexpensive way to produce pharmaceuticals. As the demand for biopharmaceuticals is expected to increase, transgenic plants have the potential to provide virtually unlimited quantities of proteins for use as tools in both human health care and the bioscience.

Many antigens have been expressed in tobacco plant and assessed for efficacy in trials with generally positive results, but, in some cases, the limiting step was the small amount of recovered protein. This could be due to the instability of the antigens in the plant cell. We developed a new strategy to accumulate and stabilized recombinant proteins in plant, which prevents targets degradation. We anchored the antigens to the cytosolic face of the endoplasmic reticulum (ER) membrane via the C-terminal region of type IV, or Tail-anchored (TA), membrane proteins. This class of polypeptides lacks an N-terminal signal sequence and reach their destination within the cell by post-translational mechanisms. They are bound to the membrane by a C-terminally located hydrophobic sequence (Transmembrane Domain, TMD), followed by a short polar region which are translocated in the lumen of the organelle of destination. They carry out a variety of essential functions (enzymatic activities, vesicle fusion - KNOLLE, AtSyr, ZIG) on the cytosolic face of membranes and their localisation is related to their function.

Candidate HIV vaccines are particularly good targets for plant-based antigens production. The major HIV antigen Nef shows promise as vaccine candidate, and we used this molecule to test our innovative startegy. With the aim to improve the amount of Nef produced in transgenic tobacco plant, we fused the tail-anchor of mammalian ER cytochrome b5 to the C-terminus of Nef. To assay the correct localisation and topology of the fusion protein, we tagged the C-terminus with a sequence containing a consensus for N-glycosylation, which occur in the ER lumen. Moreover, to recover Nef protein only, without the tail-anchor, we inserted a thrombine cleavage site between the C-terminus of Nef and the TMD. The source of HIV Nef is pSCNef 51 (Cooke et al. 1997) from the NIBSC centralised facility for AIDS reagents.

First, we evaluated the ER localisation and stability of the fusion protein, called Nef-TA, by transient expression in tobacco protoplasts and metabolic labelling. The recombinant antigen Nef-TA was expressed and N-glycosylated, indicating that the protein is targetted to the ER membrane with the correct TA topology. A pulse/chase experiment showed that Nef-TA, bound to the cytosolic face of the ER membrane, was more stable than Nef-TADTMD, a mutant in which the transmembrane domain was deleted. Now, we are producing transgenic tobacco plant expressing Nef-TA or Nef-TADTMD and we will test and compare the stability of both proteins. Than, we will evaluate the immunogenicity of recombinant HIV antigens both in crude plant extracts, as well as following purification, using C57BL/6, Balb/c or other mice in collaboration with Istituto Superiore di Sanità – Italy.

Our results indicate that the tail-anchors from type VI membrane proteins are a good tool to target and bind recombinant proteins to the ER membrane, to prevent rapid degradation and to improve the amount of product.