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Poster Abstract – F.06

TAIL-ANCHORS FROM TYPE IV MEMBRANE PROTEINS AS A TOOL TO INCREASE ACCUMULATION OF AN HIV ANTIGEN

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Transgenic plants as bioreactors have 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 to be used as tools in both human health care and bioscience.

However, the low cost and highly scalable protein biomanufacturing capacity of plants is often limited by the small amount of the recovered protein. To tackle this limitation we developed a new strategy that was expected to favour the accumulation and the metabolic stability of the recombinant protein in plant tissues. To test our strategy of plant-based antigen production we chose the Nef protein, an HIV antigen that shows promise as a vaccine candidate.

We provided the antigenic Nef molecule with an hydrophobic anchor that allows localization to and concentration of the protein on the cytosolic face of the ER membrane.

The anchoring function is provided by the C-terminal domain of the cyt b(5), a type IV, or tail-anchored (TA), membrane protein. 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 occurs in the ER lumen. Moreover, to purify the Nef protein that lacks the tail-anchor, we inserted a thrombine cleavage site between the C-terminus of Nef and the transmembrane domain region.

Stable transgenic tobacco plants expressing the Nef-TA protein or its soluble cytosolic counterpart (Nef Δ TMD) have been established. Metabolic labelling of transgenic protoplasts indicated that both the anchored and soluble Nef are stable up to 6 hours of chase. Nevertheless, the presence of the tail anchor contributes to an at least 20-fold higher increase in synthesis rate of Nef-TA compared to Nef Δ TMD. The occurrence of N-glycosylation indicates that Nef-TA is inserted into the ER membrane with the correct TA topology.

Intracellular localization of the heterologous proteins in plant cells was investigated using immunofluorescence and confocal microscopy. The distribution pattern of Nef-TA showed a typical ER staining which could be superimposed with that of the microsomal marker BiP.

The ER localization of the anchored Nef has been confirmed by subcellular fractionation experiments.

Our results indicate that the tail anchor from type IV membrane proteins represents a good tool to target recombinant proteins to the ER membrane, thus preventing rapid degradation and/or improving accumulation of the heterologous products.