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MEMBRANE INSERTION AND N-GLYCOSYLATION OF VACCINIA VIRUS A33R PROTEIN IN TOBACCO PROTOPLASTS

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Molecular farming in plants has many practical, economic and safety advantages compared with more conventional systems, and plants are becoming an attractive system for large-scale recombinant protein production. We are investigating the possibility to produce in plants antigens of Vaccinia virus, the orthopoxvirus used for vaccination in the worldwide eradication of smallpox .

Investigation on Vaccinia virus showed that the interference with the expression of any protein present in purified extracellular enveloped virus (EEV) by deletion or repression has dramatic impact on the ability of the virus to spread from cell to cell in tissue culture and form plaques. Further evidence attesting to the importance of the EEV comes from antibody studies. Several researchers have reported that antibodies to EEV proteins protect animals from viral challenge significantly more than antibodies to the intracellular mature virion.

A33R is one of EEV specific proteins and it is a highly conserved among other orthopoxviruses. It is a 23-28kDa N- and O-glycosylated type II integral membrane protein, with a short N-terminal cytosolic domain. In this study we report the transient expression of the A33R protein in protoplast of tobacco. To this aim we cloned in the vector pDHA the A33R DNA coding sequence, fused to a tag of eight amino acids at the C-terminus (FLAG epitope). Radioactive A33R can be immunoprecipitated with anti-FLAG antiserum and is still detectable after four hours of chase in a pulse-chase experiment. The protein is N-glycosylated, as indicated by a reduction in molecular mass upon pulse-chase performed in the presence of the N-glycosylation inhibitor tunicamycin. N-glycosylation indicates correct insertion into the endoplasmic reticulum membrane. Protein blot analysis suggests that the protein can form dimers (around 55kDa), similarly to what happens in animal cells.