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Poster Abstract – F.05

HIV-1 GAG PROTEIN PROCESSING IN TOBACCO CELLS

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The HIV-1 Gag protein (55 KDa) is involved in auto-assembling and formation of VLPs (Virus Like Particles) in human cells. It is composed of four distinct domains, the N-terminal matrix domain (p17), the central capsid domain (p24), the nucleocapsid domain (p7) and the C-terminal domain (p6). Two spacer peptides, p1 and p2, separate the p24/p7 and p7/p6 domains, respectively (Morikawa Y. 2003 Curr. HIV Res. 1: 1-14). In infected human cells, the Gag protein is synthesized as a precursor and converted in the mature protein by the viral protease. We expressed the Gag protein in tobacco cells using two different strategies: stable transformation of the plastidial genome and transient expression in the nucleus via *A. tumefaciens*.

In transplastomic plants, western blot analysis using a recombinant polyclonal HIV/p24 antiserum detected a strong signal of about 40 KDa and weak signals of about 55 and 24 KDa, not present in negative control plants. The 40 KDa product was detected also using a HIV/p17 antiserum. Moreover, when we incubated the protein extracts with a recombinant HIV-1 protease for different times (5-120 min), a rapid reduction of the 40 KDa band and a concomitant increase of the 24 KDa product were observed. These results demonstrate that the 40 KDa product consists of the N-terminal matrix (p17) and the central capsid (p24) domains. Hence, the presence of multiple bands is due to the processing of the Gag polypeptide by plastidial proteases similarly to what occurs with the viral protease.

In nuclear transient expression experiments, we agroinfiltrated tobacco plants (*N. tabacum* and *N. benthamiana*) with a series of *gag* binary constructs under the control of the *RbcS* promoter and 5'UTR, along with specific signal sequences for protein accumulation in different subcellular compartments (cytosol, apoplast, endoplasmic reticulum, chloroplast, and mitochondrion) and *myc*/6xHis tags. Western blot analyses carried out 3 days post infiltration (dpi) with HIV/p24 antisera did not show Gag protein accumulation in any subcellular compartment. On the other hand, RT-PCR analyses indicated that the *gag* gene was transcribed correctly, albeit at various levels with different constructs. To check whether the lack of expression was due to a particular subunit of the Gag polyprotein, we inoculated plants with constructs containing the p17, p24 or Dp17 domains (the latter including all domains except p17). Western analyses, carried out 3 dpi, detected the 24 KDa protein in plants agroinfiltrated with the p24 construct, whereas no signal was observed for the p17 construct. In the Dp17 infiltrated plants, protein analysis with HIV/p24 antiserum revealed only the p24 protein, suggesting that the native protein is quickly processed by plant proteases as already observed in *gag* transplastomic plants.

These results are useful to develop novel vectors and transformation strategies for the expression of the *gag* gene in plant cells.