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EXPRESSING HIV-1 NEF PROTEIN IN TOBACCO PLANTS

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The possibility to synthesize biopharmaceuticals (such as vaccine components) using plants paves new ways and offers solutions to some of the problems associated to traditional foreign expression systems (Ma *et al.*, 2005 Vaccine 23: 1814-18).

Modern vaccines are becoming increasingly complex, foreseeing, for example, the incorporation of multiple antigens. Approaches towards developing HIV vaccines appear to confirm this trend, with a combination of candidate antigens being recommended by several groups.

Plant expression of a number of these candidates has already been achieved, including HIV-1 gp120 envelope glycoprotein, p24 core protein and the regulatory Tat protein. Both regulatory and accessory HIV proteins are currently regarded as promising targets for vaccine development as they could provide further protective efficacy in combination with viral structural proteins. In this regard, HIV-1 accessory Nef protein is considered a promising target (Robert-Guroff 2002, DNA Cell Biol. 21: 597-8).

Nef is incorporated into viral particles and expressed in the early stage of infection both in the cytoplasm and on the cell membrane of virus-infected cells. Nef interacts with multiple host factors in order to optimise the cellular environment for virus replication. Its critical role in pathogenesis is demonstrated by the fact that the infection with *nef*-defective HIV strains dramatically decreases the rate of disease progression in seropositive individuals (Tobiume *et al.*, 2002, J.Vir. 76: 5959-65). Moreover, Nef could be an important component for CTL-based HIV-1 vaccines, therefore immune responses directed against this viral protein could help to control the initial steps of viral infection and reduce viral loads and spreading (Robert-Guroff 2002, DNA Cell Biol. 21: 597-8).

Studies of genetic characterization of nef gene showed that two proteins could be translated *in vitro* and expressed in mammalian cells: a full-length N-terminal myristoylated form of 27 kDa (p27) and a truncated form of 25 kDa (p25) lacking the first 18 amino acids (Kaminchik *et al.*, 1991, J Vir. 65: 583-8).

To explore the possibility of plant Nef expression, a number of different constructs have been used to generate independent transgenic lines of *Nicotiana tabacum* cv. Petit Havana. A number of transgenic lines expressing at high levels both p27Mut and p25 Nef isoforms have been identified. Characterization of these transgenic lines is currently being performed.

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