The effect of expressing an anti-HIV lectin, Griffithsin, in different plant cellular compartments

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HIV in Africa

• Sub-Saharan Africa remains the region most heavily affected by HIV
• Majority of new infections from heterosexual contact in women
• The cervical/vaginal mucosa is the main entry point of HIV in women (WHO/UNAIDS: Report on Global AIDS Epidemic. May 2006b and December 2009)
Microbicides

• A **microbicide** is any compound or substance whose purpose is to reduce the infectivity of microbes, such as viruses or bacteria

• Mode of action
  - Destabilizing virus structures
  - Preventing docking/entry
  - Preventing HIV replication
  - Combination drugs
  (Stone and Jiang, ww.thelancet.com Vol 368 August 5, 2006)

• Lectins have a unique interaction with the virus and presents a novel approach to target the virus

• Need for cost effective production for distribution to poorer populations
Griffithsin

- Griffithsin (GRFT) was isolated from the red algae *Griffithsia* sp. (Mori *et al.*, 2004)

- GRFT is effective in low concentrations against both primary as well as laboratory-adapted HIV isolates of different clades

- GRFT is very stable and potent in cervical/vaginal lavage fluid

- GRFT did not show toxicity to the mucosal epithelial cells

- The molecular target is the gp120 envelope glycoprotein

- GRFT has multiple mannose binding sites

*Ziolkowska *et al.*, 2006
Advantages of plant production systems

- Protein processing tools
- Versatile
- Short turnover period
  - Stable transformation
  - Transient expression
- More cost effective
- Ease of scale up
- Commercially competent
What to consider

- Type of molecule
- Molecular tools
- Plant host
- Subcellular targeting
- Purification strategies
Aim of the study

Investigate the effect of subcellular targeting of GRFT in tobacco on expression levels and plant cell viability

- Integration vector
- Deconstructed viral vector
Subcellular location

- Nucleus
- Nucleolus
- Endoplasmic reticulum
- Vacuole
- Chloroplast
- Cytoplasm
- Golgi
- Mitochondria
- Cell wall
- Cell membrane
- Apoplast
- Endoplasmic reticulum
- Vacuole
- Chloroplast
- Cytoplasm
- Apoplast
Tobacco integration vectors- pTRA

- Expression under the control of the 35S CaMV promoter.
- Targeting to Apoplast, Cytosol, Chloroplast and retention in the Endoplasmic Reticulum (ER).
Tobacco deconstructed viral vectors - Icon

3’ GRFT

5’ Cytosol

5’ Apoplast

Recombinase
Assembly of viral pro-vector modules in plants

Marillonnet et al., 2003
GRFT integration vector infiltrated

Expression in mg/kg fresh leaf weight

- 6 days post infiltration
- 8 days post infiltration
- 12 days post infiltration

Cell compartment

- Apoplast-GRFT
- Cytosol-GRFT
- Chloroplast-GRFT
- Endoplasmic reticulum-GRFT
GRFT deconstructed viral vector infiltrated

Apoplast

Cytosol

6 dpi
8 dpi
12 dpi

Wt Infiltrated

Expression in mg/kg fresh leaf weight

GRFT cytosol

6 days post infiltration
8 days post infiltration
12 days post infiltration

Wt
Infiltrated
Coomassie and Immunoblot of GRFT expressed in the cytosol

- Coomassie indicates expression of GRFT and MW
- Western blot analyses confirm exclusive recognition by antibody and MW
### Efficacy data

<table>
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<th><em>N. benthamiana</em> produced GRFT ID$_{50}$</th>
<th>Untransformed Tobacco IC$_{50}$</th>
<th><em>E. coli</em> GRFT IC$_{50}$</th>
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- **IC$_{50}$ (ng/mL)** and ID$_{50}$ (given as a ratio) needed to neutralize virus
- **IC$_{50}$** is the concentration of substance that provides 50% inhibition
- **ID$_{50}$** the dose that will infect 50% of the experimental group
- **IC$_{50}$** of Nevirapine = 10 ng/ml
Conclusions

- Different expression levels were observed for the different vector systems. The Icon vector system exhibited expression levels more than 15 fold higher than the pTRA vector system.
- Targeting to different cell compartments affected yield and protein accumulation; in both vector systems
  - Apoplast associated with toxicity as reflected by leaf tissue death in both vector systems
  - Some accumulation observed (40mg/kg) prior to cell death
- *N. benthamiana* produced GRFT was detected by the polyclonal antibody and had the same molecular weight size as *E.coli* produced GRFT. Both monomeric and dimeric forms of GRFT were detected.
- GRFT from *N. benthamiana* recognized viral coat protein gp120 in ELISA analysis and was able to neutralize HIV sub-type C in vitro.
- Commercially viable levels of expression obtained with both systems 30 mg/kg fresh weight and 500 mg/kg for pTRA and Icon respectively (in plant systems levels of <100mg/kg considered potentially commercially viable, Evangelista *et al.*, 1998).
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Thank You