Production of antigens and antibodies in plants: alternative technology?

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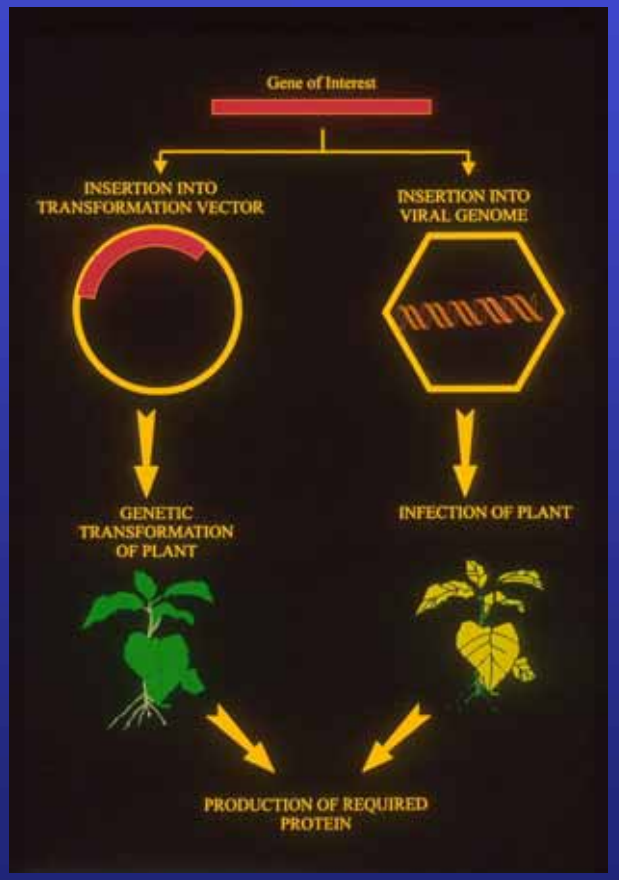
Why use Plants as Biofactories?

- Produce large biomass
- Require minimal inputs
- Keep themselves sterile
- Any product will not be contaminated with an animal pathogen

“Classical” Methods for Expression of Foreign Proteins in Plants

- Creation of lines of plants transgenic for gene of interest
- Use of modified viral genomes for introduction of gene of interest



Advantages of Transgenic Approach for Expression

- Introduced sequence is heritable and true-breeding lines of plants can be created
- Transgene present in every cell of plant.
- Site of expression can be controlled by use of tissue-specific promoters
- No limit on size or complexity of proteins which can be expressed

Disadvantages of Transgenic Approach for Expression

- Transformation/regeneration can be difficult and time-consuming especially for crop species
- Large variability in properties of resultant transgenic plants making evaluation of different constructs difficult
- Often get only low level of expression of inserted gene

Advantages of Plant Virus Vectors for Expression

- Viruses multiply in their hosts and therefore high level of expression is expected.
- Genetic modification of viral genomes is straightforward and quick.
- Infections can be easily passaged to fresh plants for bulking up material.
- Particles are stable and have defined structure.

Disadvantages of Plant Virus Vectors for Expression

- Limits on size of sequence that can be inserted into a viral genome.
- Multiple rounds of replication may lead to the accumulation of mutations within inserted sequence.
- If a vector based on a wild-type virus is used, there may be problems of containment.

Alternative methods

- Chloroplast transformation
  + Increased copy number of inserted genes; no pollen transfer
  - can be difficult to obtain homoplasty; no glycosylation
- Transient Agrobacterium-based expression
  + Very quick; can test many different constructs – high throughput
  - Problems of scale up; need efficient method of infiltration
- Combined transgene/virus systems
  + Potentially high level expression; can use disabled virus for bio-containment
  - Requires the production of transgenic lines

Choice of host

- Whole food plant (e.g. potato, soya, maize)
- Whole non-food plant (e.g. tobacco, Arabidopsis)
- Unusual plants (Duckweed, moss, algae)

Containment?

- Physical e.g. in containment greenhouses, tissue culture vessels. Problems of expense.

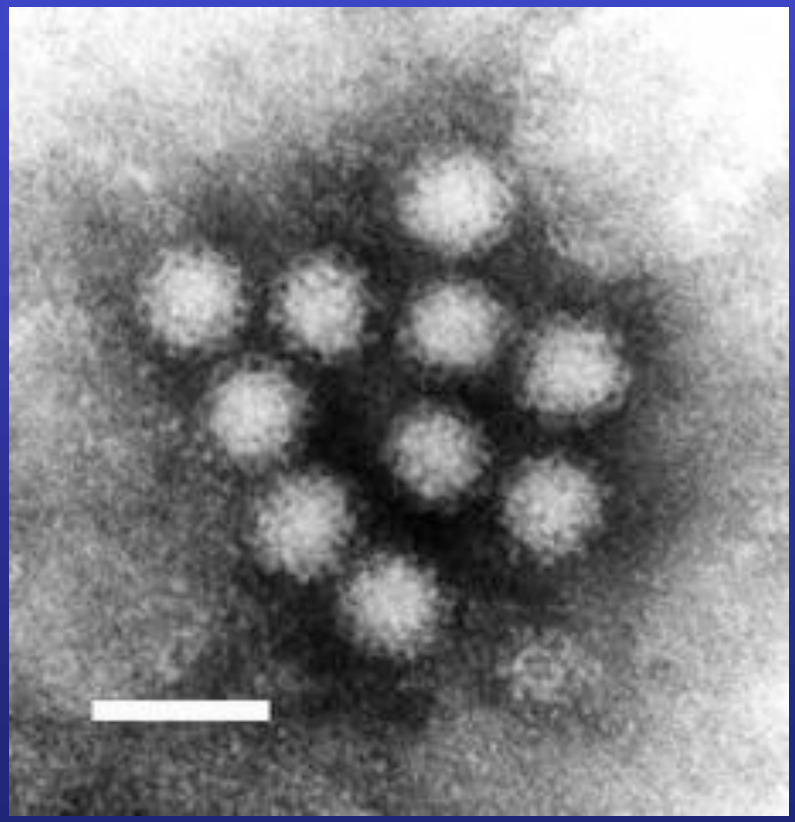
- Biological e.g. chloroplast transformation o prevent pollen transfer, use of disabled viral vectors

Examples

- Norwalk virus in transgenic potatoes

Production of a vaccine against norwalk virus

- Small RNA virus which causes intestinal infection
- Results in diarrhoea and vomiting (*Aurora*)
- Cannot grow virus in culture
- No vaccine or treatment available



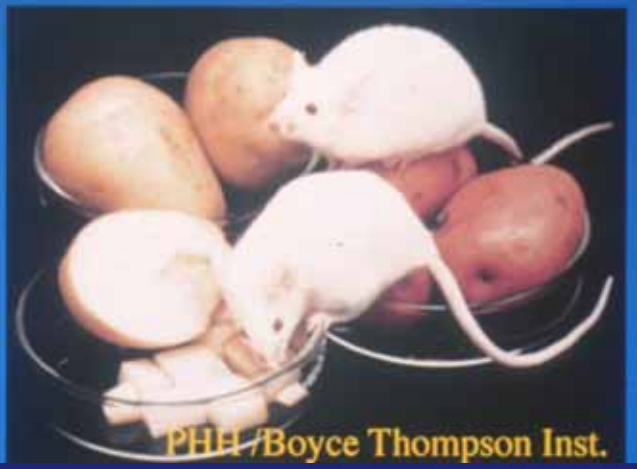
Use of plants to produce oral vaccines

Testing the Concept of Oral Vaccine Production in Plants

1. Introduce gene encoding antigenic protein of human pathogen into plant cells
2. Regenerate plants which produce the foreign antigenic protein (subunit vaccine)
3. Grow plants to maturity and harvest edible tissue

4. Feed to test animal and analyze immune response

Finding: Mice were orally immunized by simply feeding the transgenic potatoes.



Results

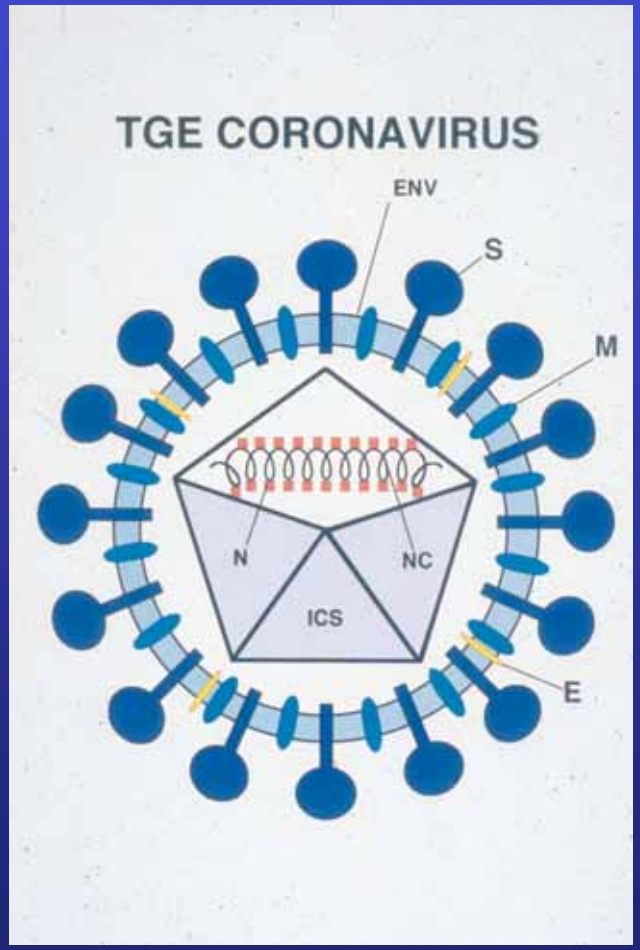
- Expression detected in plants
- Expressed protein forms NV particles
- Plant-derived particles stimulate mucosal immune response when orally administered to mice
- When peeled raw potatoes were fed to human volunteers increased levels of NV antibodies were found.

Examples

- Norwalk virus in transgenic potatoes
- Production of anti-TGEV SIP in cowpea using a viral vector

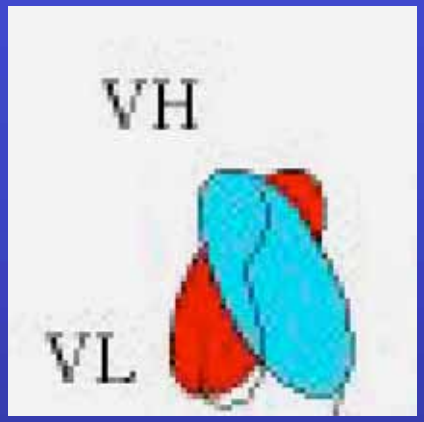
What is TGEV?

- TGEV is a coronavirus which infects via the enteric tract.
- Newborn animals are protected by passive immunisation with antibodies in milk
- Oral administration of antibodies may be an effective therapy IF antibodies can be produced at sufficiently low cost.

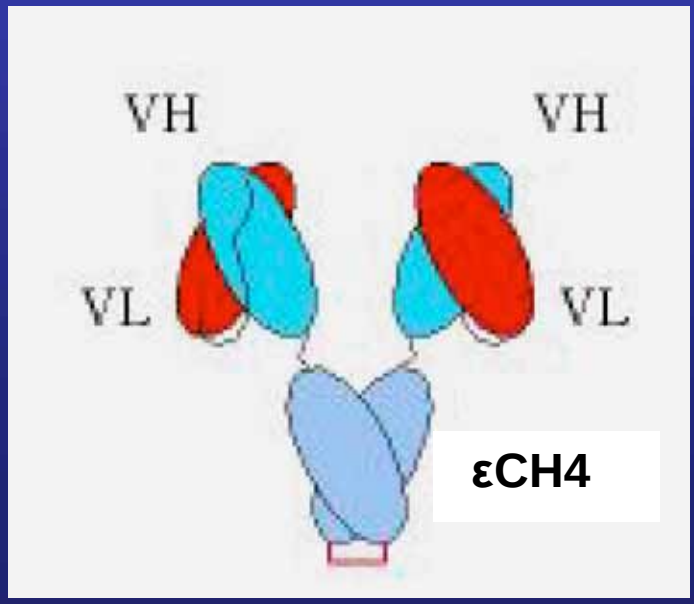


Structures of scFv and SIPs

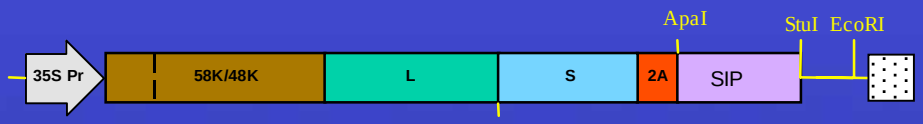
scFv's have advantage that they are small molecules (approx. 250 amino acids). However, they are monovalent and therefore have low avidity.

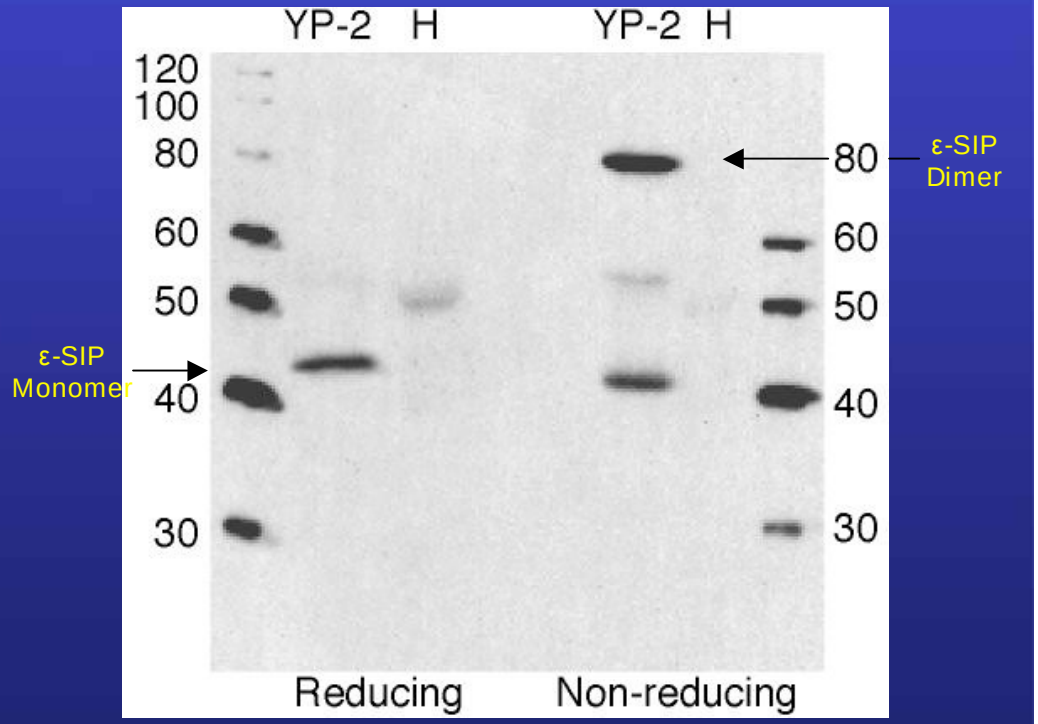


SIPs are larger molecules (approx . 380 amino acids) but have the advantage that they can dimerise to increase avidity. SIPs containing εCH4 dimerise rapidly due to C-terminal Cys.



ε-SIP expression in Cowpea





In vivo protection

in vivo protection assay:

100g plant tissue

↓

100 ml protein extract

↓

lyophilised

↓

mixed with milk

↓

fed challenged newborn piglets

↓

determine virus titres
in gut and lung

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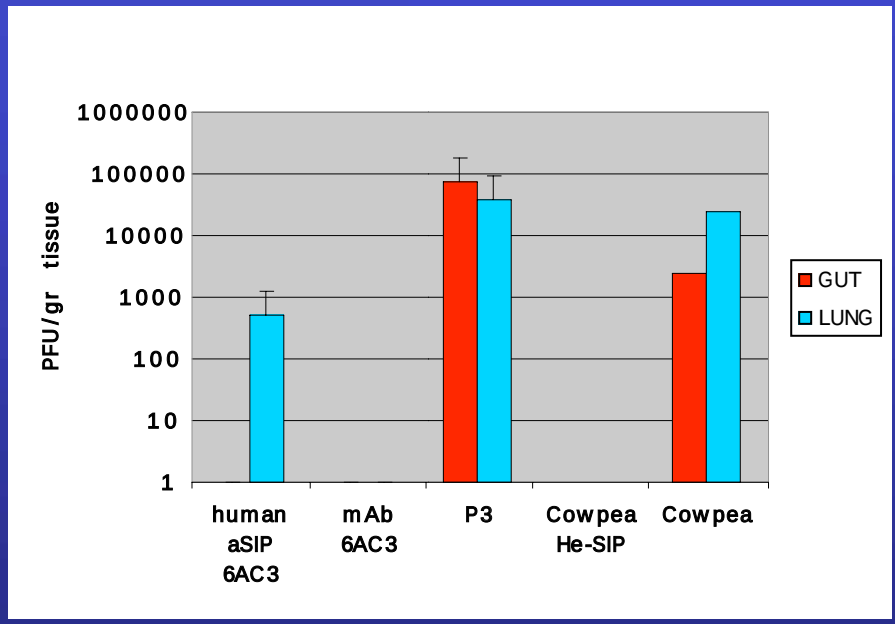
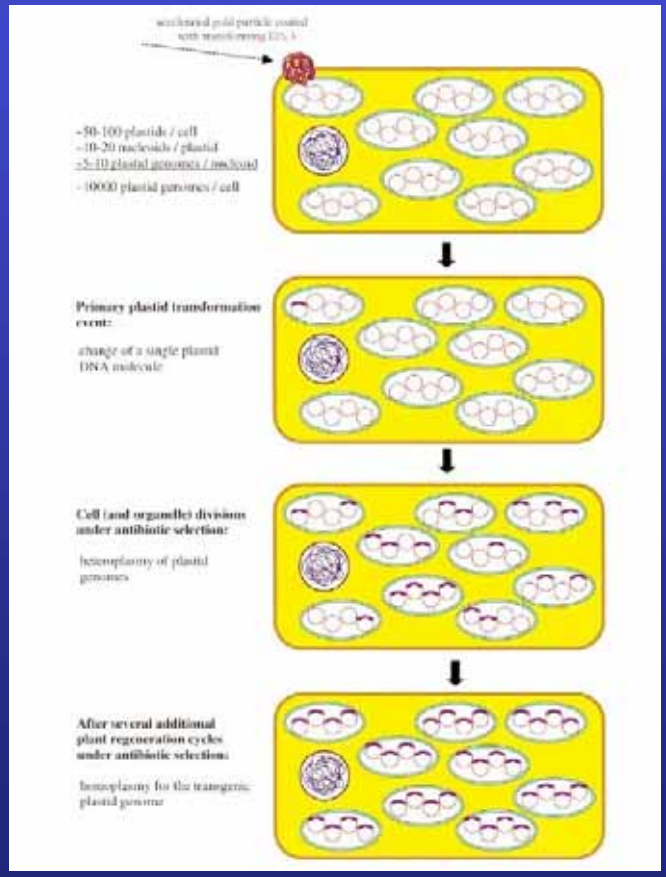


Examples

- Norwalk virus in transgenic potatoes
- Production of anti-TGEV SIP in cowpea using a viral vector
- Production of cholera toxin in chloroplasts

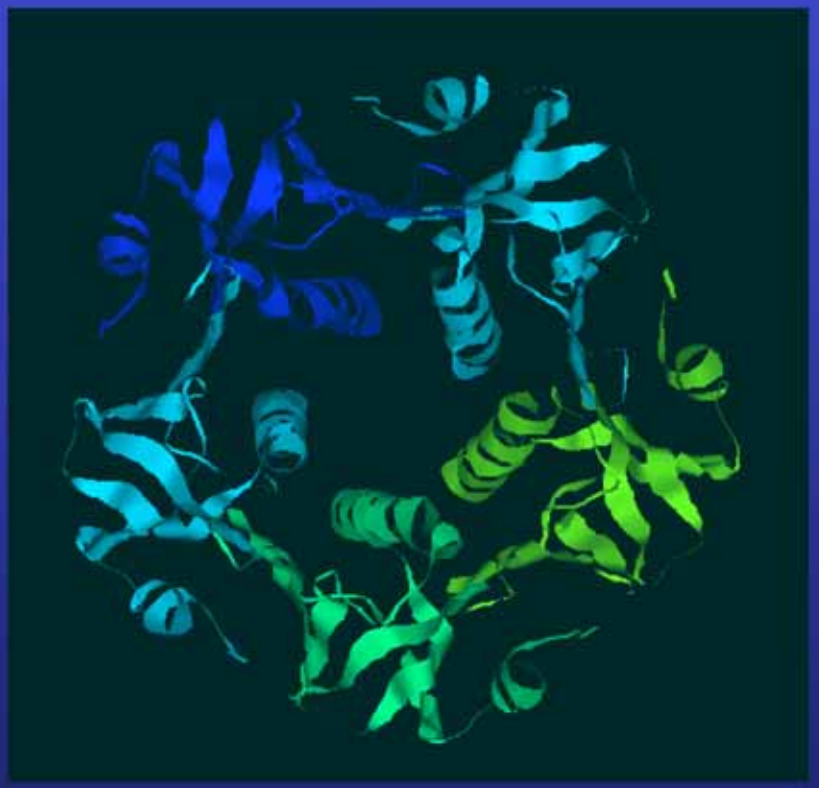
Chloroplast transformation

- Usually achieved by biolistic bombardment
- Integration into chloroplast genome is by homologous recombination
- Plants selected by rounds of antibiotic selection



Expression of cholera toxin B subunit

- B subunit targets toxin to ganglioside GM1 in gut
- Non-toxic without A subunit
- Pentamer of 11.6kDa protein subunits
- Used to orally immunise against cholera



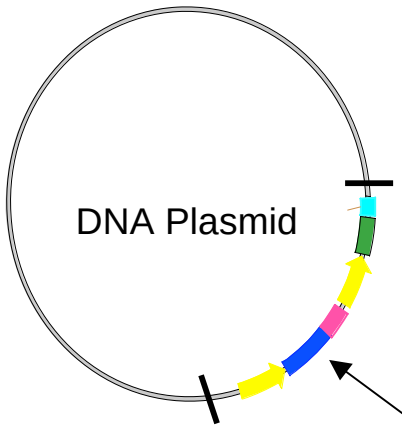
Expression of CT-B from chloroplasts

- Tobacco chloroplasts transformed with CT-B sequence
- Expressed protein was shown to oligomerise to give pentamers
- Levels reached at least 4.1% soluble leaf protein
- Expressed protein shown to bind to GM1 *in vitro*

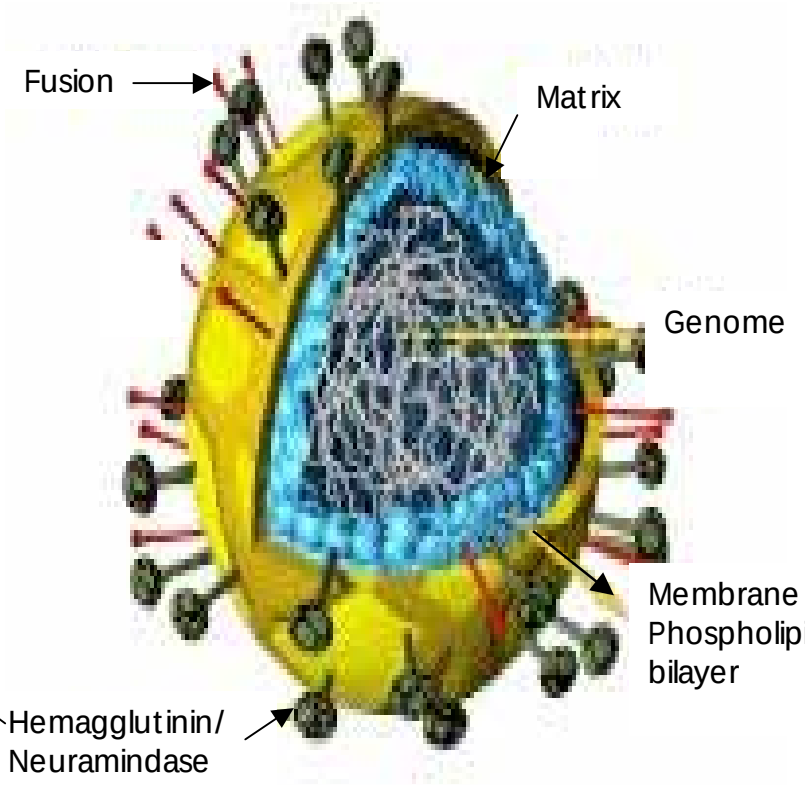
Examples

- Norwalk virus in transgenic potatoes
- Production of anti-TGEV SIP in cowpea using a viral vector
- Production of cholera toxin in chloroplasts
- Production of Newcastle Disease vaccine in cell culture (Dow AgroScience)

Design of a Plant cell produced antigen



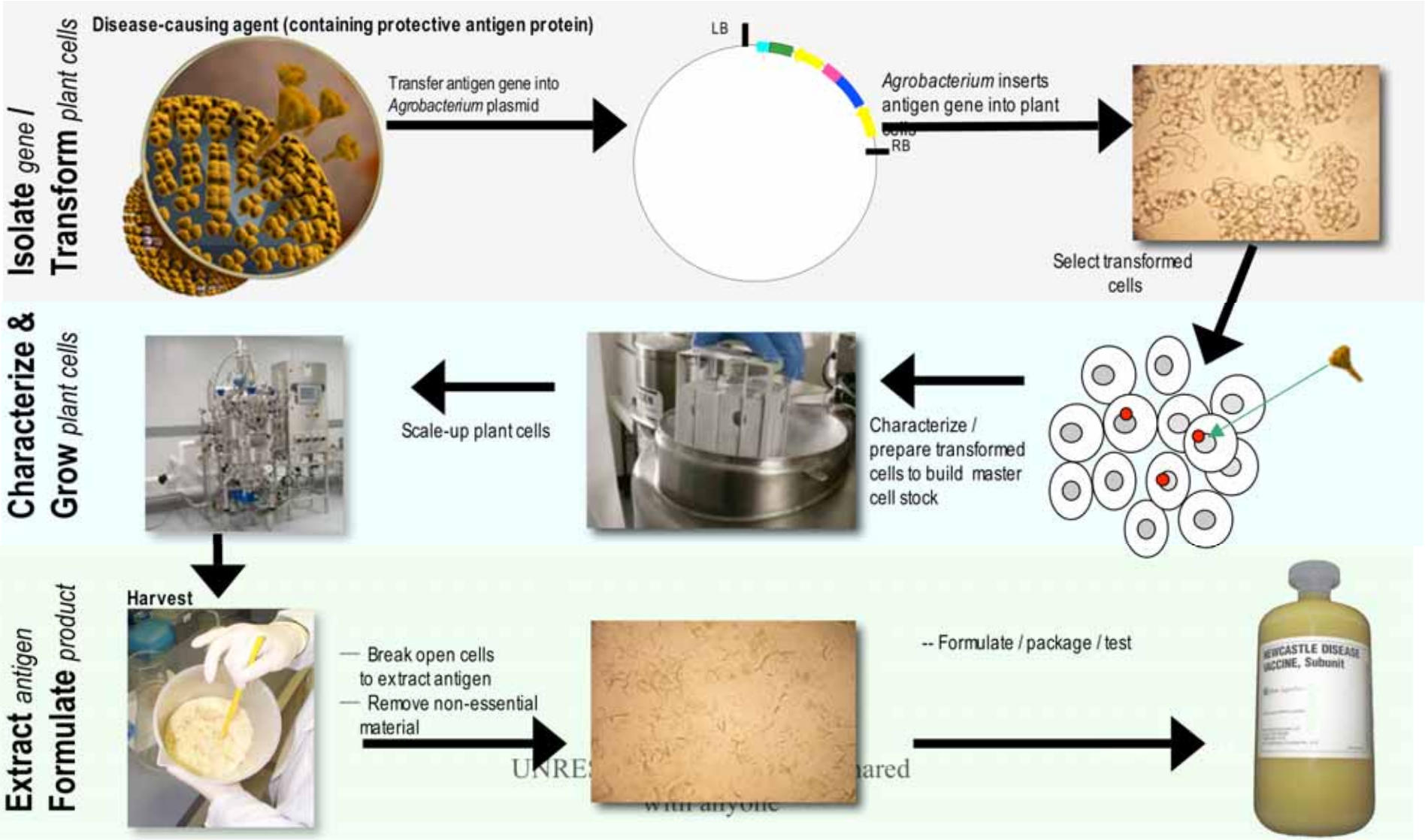
Plasmid containing HN gene for the protective antigen prepared for insertion into plant cells



Newcastle disease virus (NDV)

Russell Nightly Media

Concert™ Plant-Cell-Produced Production System



Prospects

- The science behind plant-expressed pharmaceuticals has developed greatly.
- The granting of a license to DowAgrosciences for NDV vaccine is a breakthrough
- There is renewed interest in Plant-based vaccines e.g. FP6 PharmaPlanta