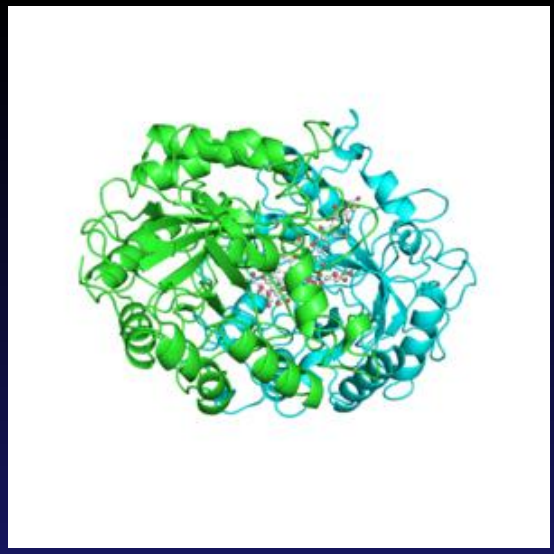
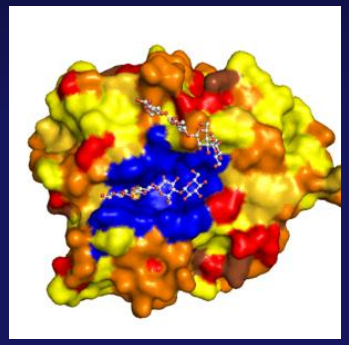
Protein expression systems in plants: engineering proteins in the greens





Heraklion 2019

Today's tutor

https://www.imbb.forth.gr/en/research-en/plant-molecular-biology/item/4119-panagiotis-n-moschou

FORTH
INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGY

ΕΥΡΩΠΗ

SLU

Learning outcomes

- ✓ Appreciate plants as a protein expression system
- ✓ Strategies and frameworks for protein expression in plants

Keywords

- Algae
- Chloroplasts
- Agrobacterium
- Transient and stable expression
- Transgenes
- Viruses

What you already know about plant protein expression?

Which are the major challenges? Any personal experiences with plant expression systems you would like to share? What questions do I have already about this topic?

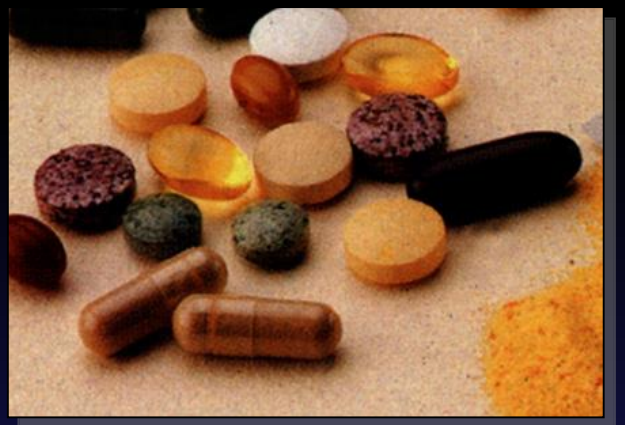
Expression systems

Expression Systems	Bacteria	Yeasts	Cultured Mammalian Cells	Animals	Plants	Microalgae
Protein folding accuracy	Low	Medium	High	High	High	High
Glycosylation	None	Incorrect	Correct	Correct	Minor Differences	Minor Differences
Product quality	Low	Medium	High	High	High	High
Protein yield	Medium	High	High	High	High	High
Production scale	Limited	Limited	Limited	Limited	Worldwide	High
Production time	Short	Medium	Long	Long	Long	Short
Scale-up cost	High	High	High	High	Medium	Low
Overall cost	Medium	Medium	High	High	Low	Low
Contamination risk	Endotoxins	Low	High	High	Low	Low
Safety	Low	Unknown	High	High	High	High
Storage cost	Moderate	Moderate	Expensive	Expensive	Inexpensive	Low
Distribution	Medium	Medium	Difficult	Difficult	Easy	Very easy
Reproduction	Easy	Easy	Difficult	Medium	Easy	Very easy

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4926494/

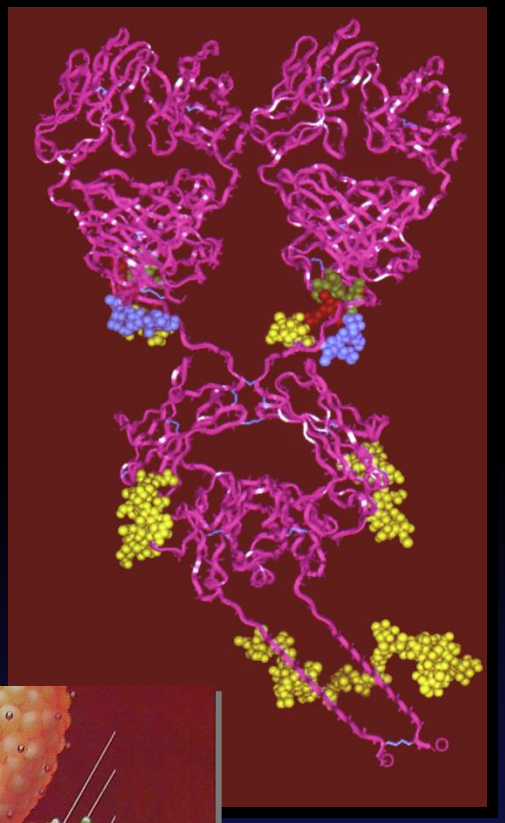
Why do we need more?

- Current working concept of biothreat defense: Create Strategic Reserves of Therapeutics and Vaccines against known biothreat agents
- Limitations - large number of agents, multiple strains, ability to mutate or modify a strain to make it resistant to treatment, long term instability of therapeutics in the reserve, and overall cost

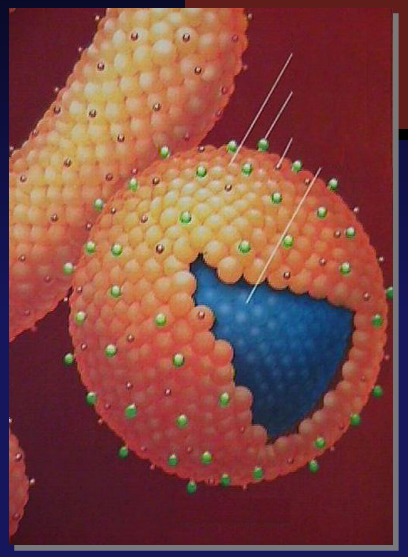


Plant-Made Pharmaceuticals (PMPs)

1989 Hiatt, A., Cafferkey, R. and Bowdish, K. Production of Antibodies in Transgenic Plants. *Nature* 342: 76-78.



1992 Mason, H.D., M.-K. Lam and C. J. Arntzen. Expression of hepatitis B surface antigen in transgenic plants. Proc. Natl. Acad. Sci. USA 89:11745-749.



Human Clinical Trials (Vaccine in Food)

- ✓ Plant Engineering:
- ✓ Choose a plant which is facile for protein expression
- ✓ Use a plant that can be eaten uncooked

Regulatory:

- ✓ Pre-clinical studies with mice
- ✓ Vaccine is only a "food additive"

For Vaccines, Five Human Clinical Trials

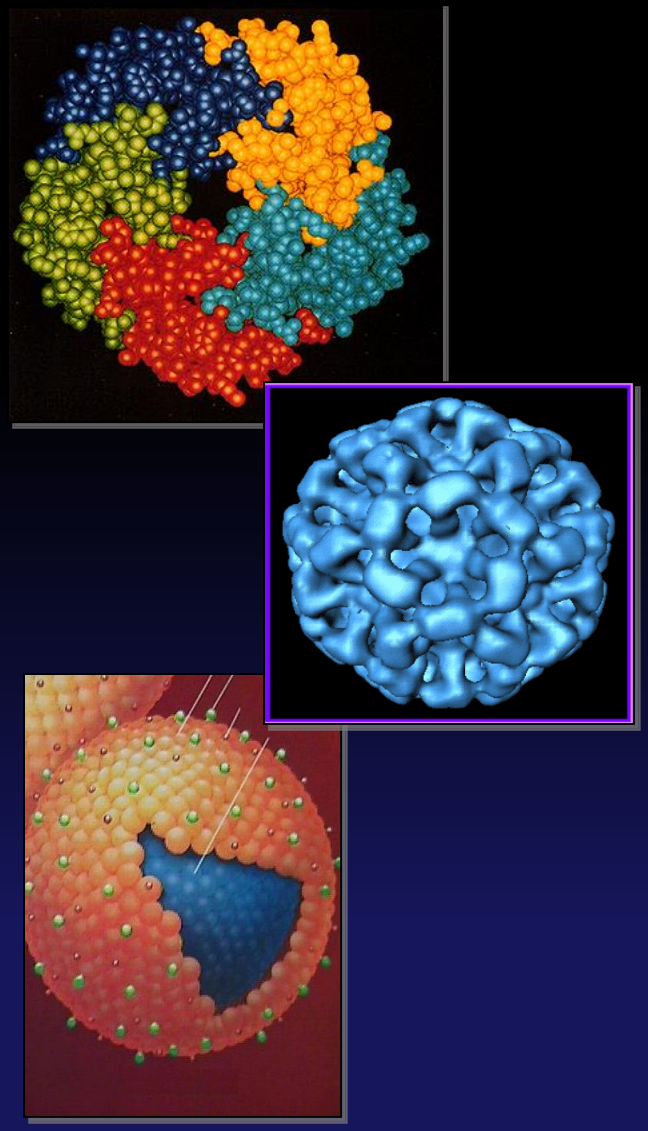
Three trials used raw potatoes

- **Tacket, C.O., Mason, H.S., Losonsky, G., Clements, J.D., Levine, M.M., C.J. Arntzen.** 1998. Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. Nature Medicine, 4:607-609.

- **Tacket, C.O., H.S. Mason, G. Losonsky, M.K. Estes, M.M. Levine, C.J. Arntzen**. 2000. Human immune responses to a Novel Norwalk virus vaccine delivered in transgenic potatoes. The Journal of Infectious Diseases. 182:302-305.

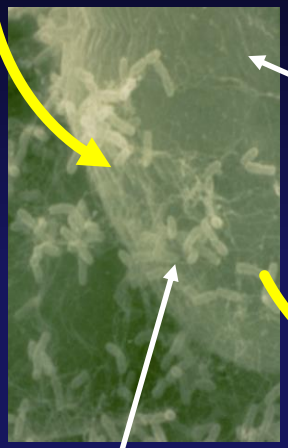
- **Thanavala, Y., Mahoney, M., Pal, S., Scott, A., Richter, L., Natarajan, N., Goodwin, P. and H.S. Mason**. 2005. Immunogenicity in humans of an edible vaccine for hepatitis B. PNAS. 102, 3378-3382.

The others used corn seed or lettuce

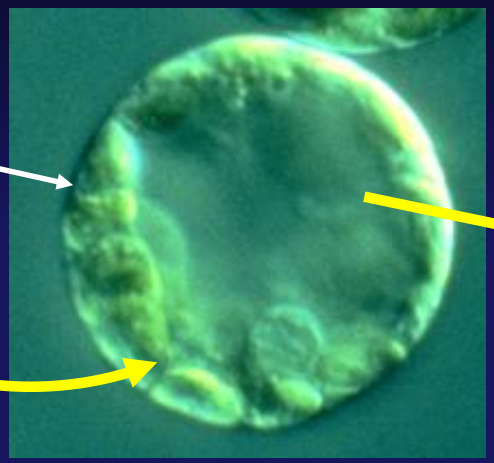


Plant-derived Pharmaceutical Protein Production

- Design a gene for proteins(s) of choice and introduce it into a plant expression vector (example: *Yersinia* antigens).
- Produce the protein using one of two expression systems: transient expression (non-integrating vector) or stable transgenic plants (shown here).

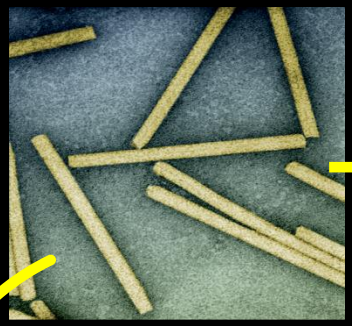


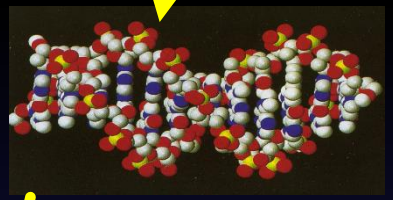
Plant Cell



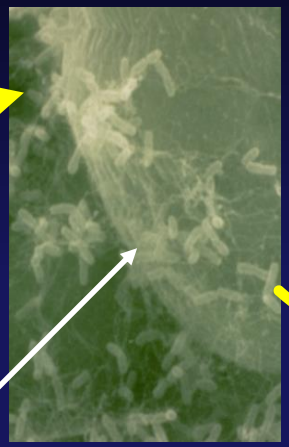
Agrobacterium

Transient Gene Expression in Plants





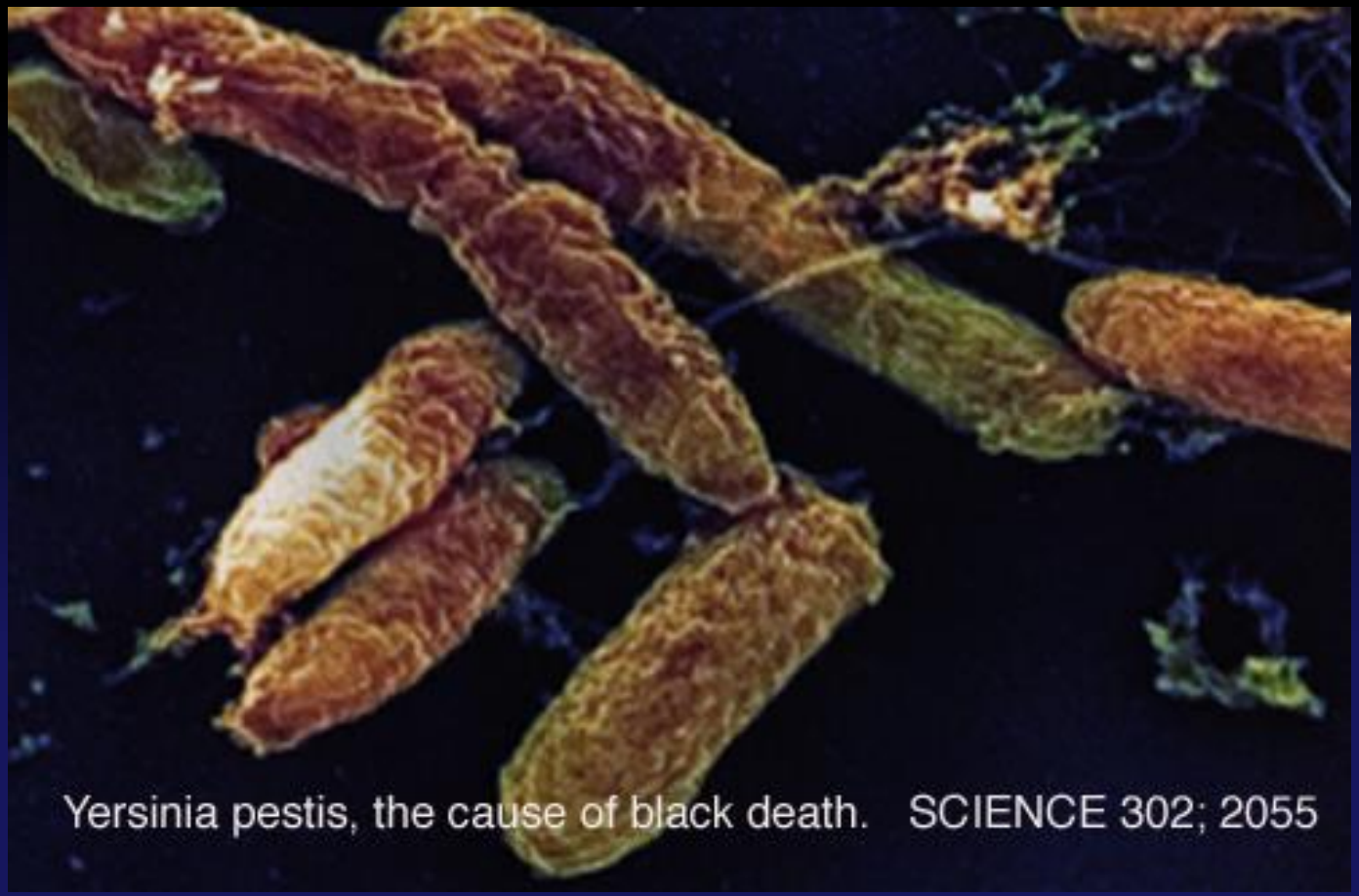
- (#1) Engineer desired gene into plant virus; protein expression as a by-product of viral replication.
- (#2) Convert RNA virus to DNA sequence and move into a delivery vector such as *Agrobacterium;* infiltrate *Agrobacterium* into leaves to express RNA from the DNA sequence and achieve "deconstructed virus*" replication.



(*ICON Genetics' magnICON® vectors as one major example)

Case Study: Plague Vaccine Vaccines

US Army Research supported a study of "Plant Production of Vaccines for Protection Against Biowarfare Agents"



Plague Vaccine Research

- 100 plants will yield a gram of purified vaccine (*ie*., 75,000 doses)
- Transient expression using “deconstructed virus” required 12 days from infection to harvest

Plague Vaccine Candidates

Gram quantities of *Yersinia pestis* antigens F1, V and an F1-V fusion protein were purified for injection delivery.

Perry et al., PNAS, Jan. 24, 2006

The antigens were successfully used to immunize guinea pigs, which were protected from *Yersinia* aerosol challenge trials at USAMRIID. Preliminary studies show that we can develop an oral delivery formulation, at least for boosting doses.

Case Study 2: Organophosphate nerve-agents countermeasures

Recent history of "successful" use of nerve agents by rogue states and terrorist organizations

Organophosphates

Sarin, Soman, Tabun, VX

Malathion, Parathion, Diazinon, Fenthion, Dichlorvos, Chlorpyrifos

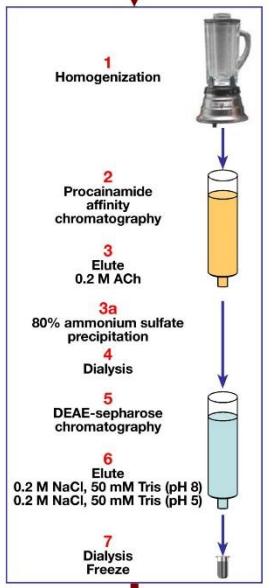
Organophosphate toxicity occurs by inhibition of acetylcholinesterase

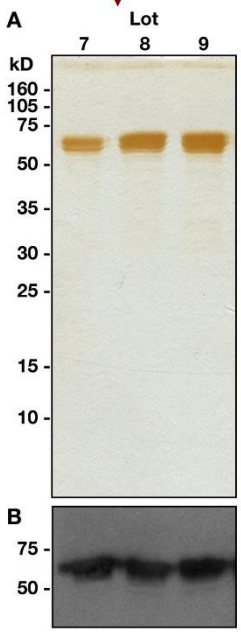
Therapeutic strategy: utilize human AChE as a molecular "sponge" to bind nerve gas agents

Validation: purified AChE from blood is functional

Plants will "biomanufacture" human AChE

Transgenic *N. benthamiana* plants expressing ACHE-R_{SEKDEL} at the ASU Pharming Greenhouse.





A. Silver-stained SDS-PAGE

B. Western blot

Purification of AChE-R_{SEKDEL}

Lot	Fresh weight (kg)	Yield (U)	Spec. Activity (U/mg prot.)
7	1.5	4,191	3,794
8	1.7	8,430	4,495
9	2.7	10,865	4,614
Total:	5.9		
	Total:	23,486	
		Average:	4,301

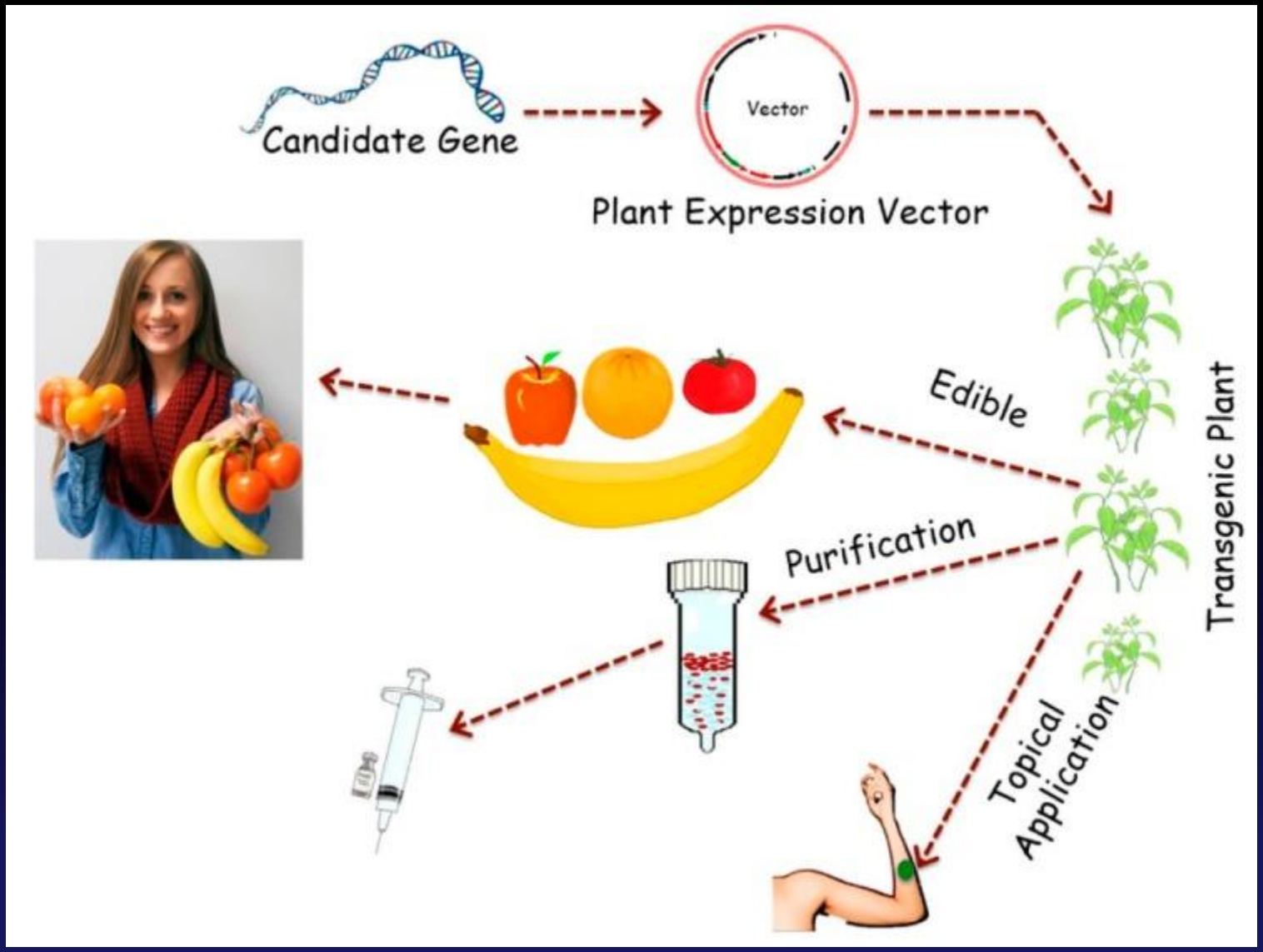
Figure 2. Purification of plant derived AChE-R_{SEKDEL}. Three lots were analyzed further by SDS-PAGE and western blotting.

Plants were shown to produce human AChE

- Active
- "Human" kinetic properties
- Inhibitor binding mimics human enzyme

Current research: expression of BChE in native and form

https://www.nature.com/articles/srep13247

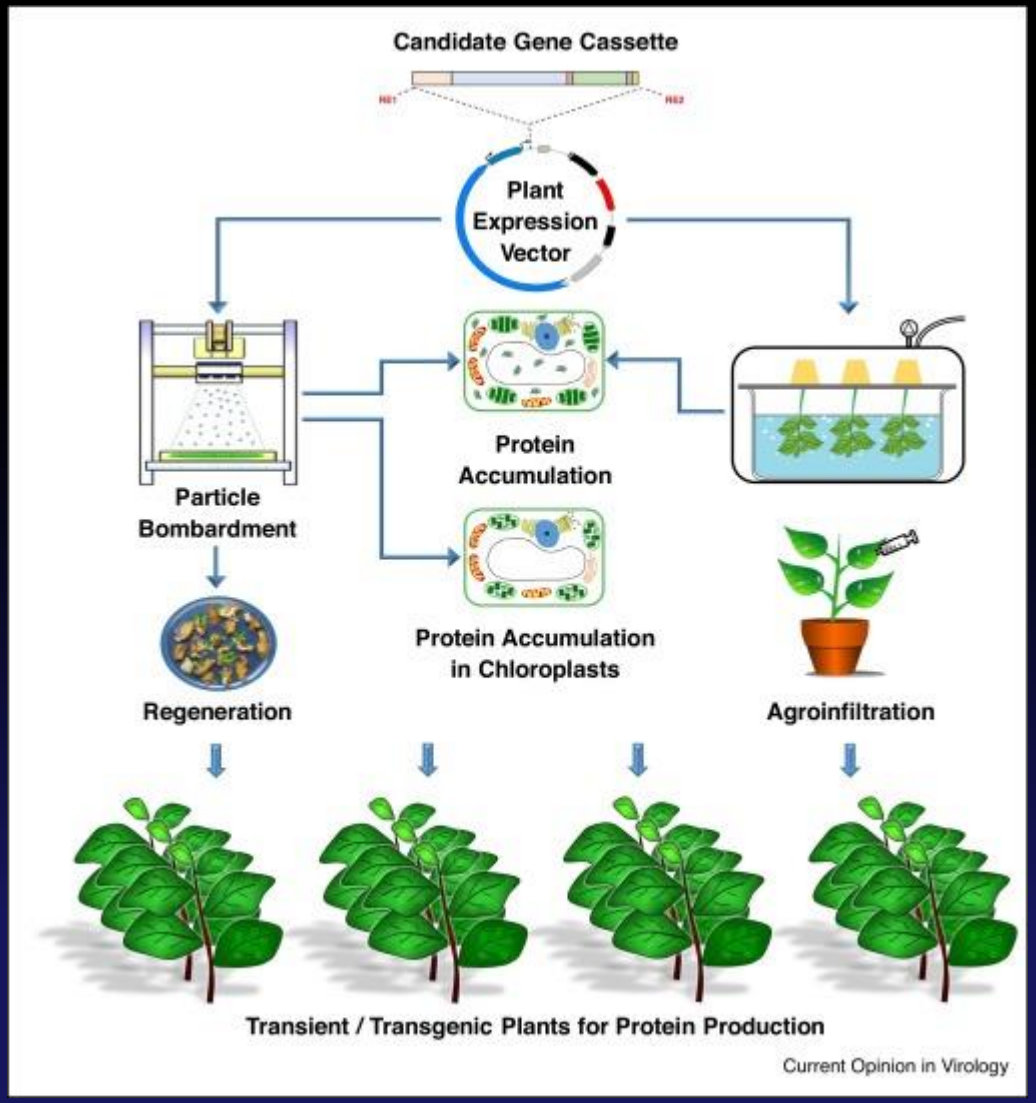

Candidate Gene
Vector
Plant Expression Vector
Transgenic Plant
Edible
Purification
Topical Application

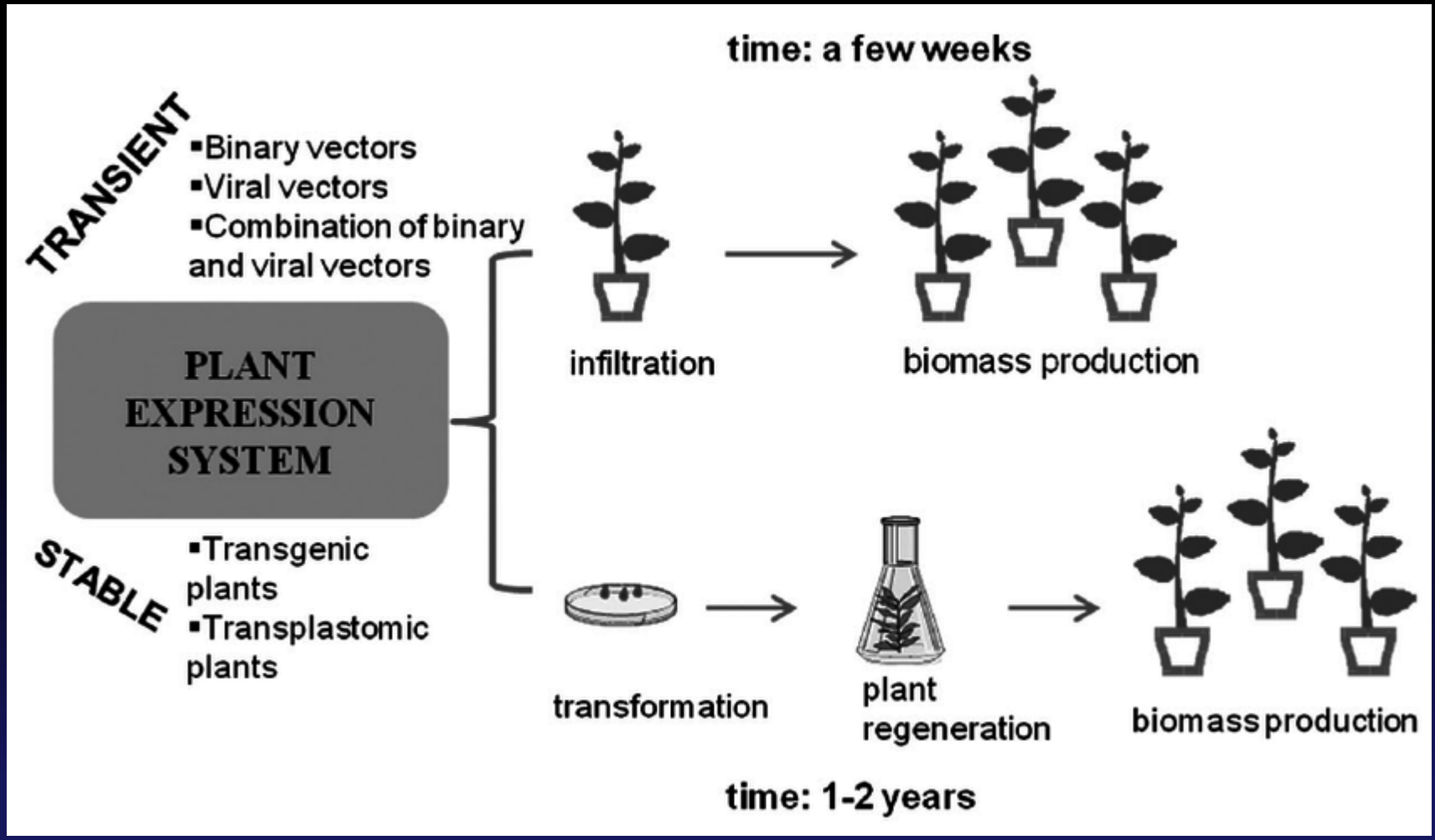
cGMP Manufacturing Facility

Plant production advantages:

- Capital cost avoidance
- Scalable production

Dual Use Facility -- Exploratory infectious disease studies to maintain expertise and advance the technology


Candidate Gene Cassette
Plant Expression Vector
Particle Bombardment
Protein Accumulation
Protein Accumulation in Chloroplasts
Regeneration
Agroinfiltration
Transient / Transgenic Plants for Protein Production
Current Opinion in Virology


time: a few weeks
TRANSIENT
▪Binary vectors
▪Viral vectors
▪Combination of binary and viral vectors
PLANT EXPRESSION SYSTEM
infiltration
biomass production
STABLE
▪Transgenic plants
▪Transplastomic plants
transformation
plant regeneration
biomass production
time: 1-2 years

Viruses

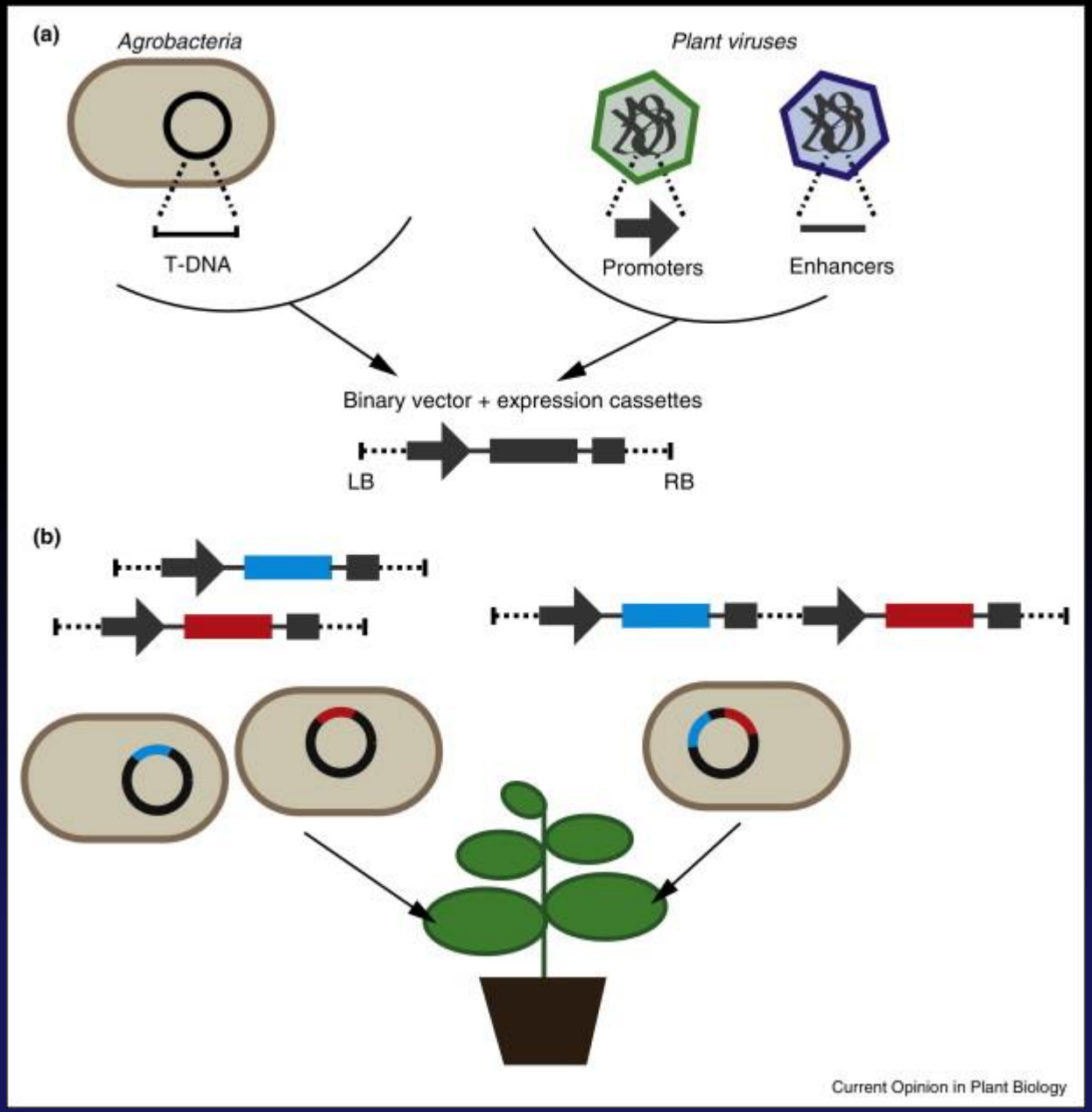
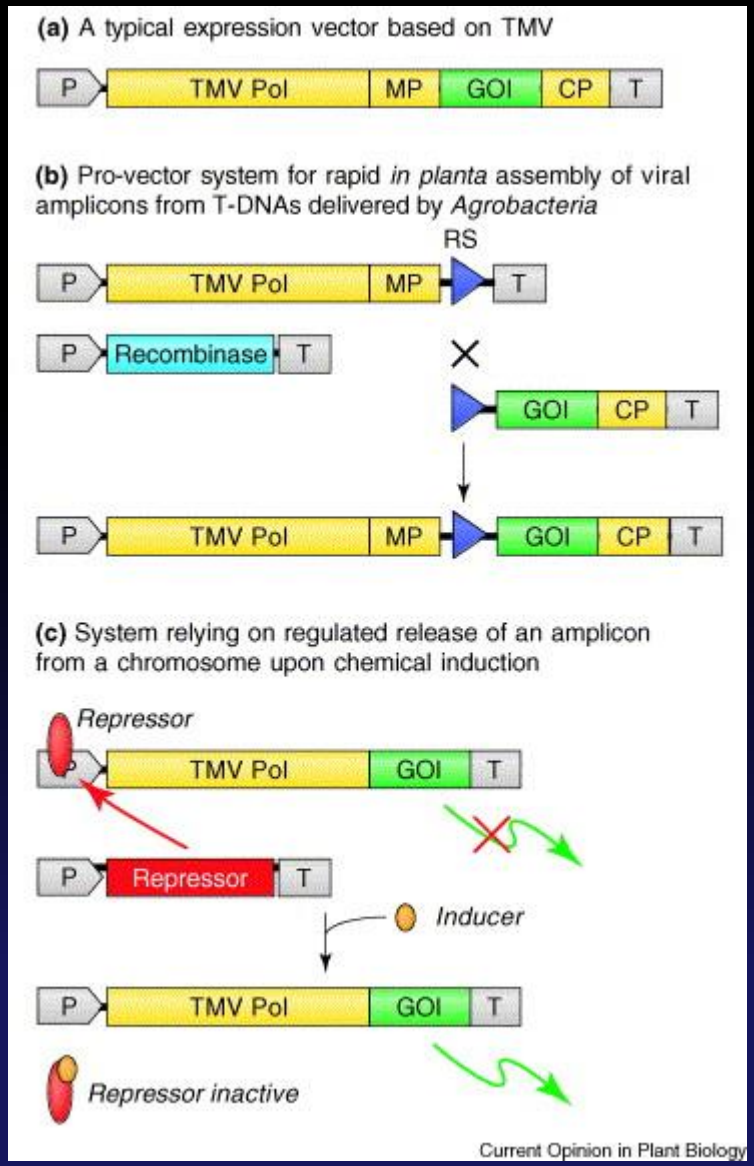


Figure 1. Development of transient expression systems for use in plant-based synthetic biology. **(a)** Disarming and reprogramming of the Agrobacteria tumour-inducing plasmid was combined with plant viral regulatory sequences to give binary vector systems harbouring high-yielding expression cassettes. **(b)** Co-expression may be achieved via co-infiltration of multiple Agrobacteria cultures containing separate binary vectors or cultures possessing single vectors harbouring multiple expression cassettes.

The deployment of suppressors of gene silencing, such as the popular P19 from Tomato bushy stunt virus (TBSV), to reduce post-transcriptional gene silencing

https://www.sciencedirect.com/science/article/pii/S1369526614000193

Viral vectors

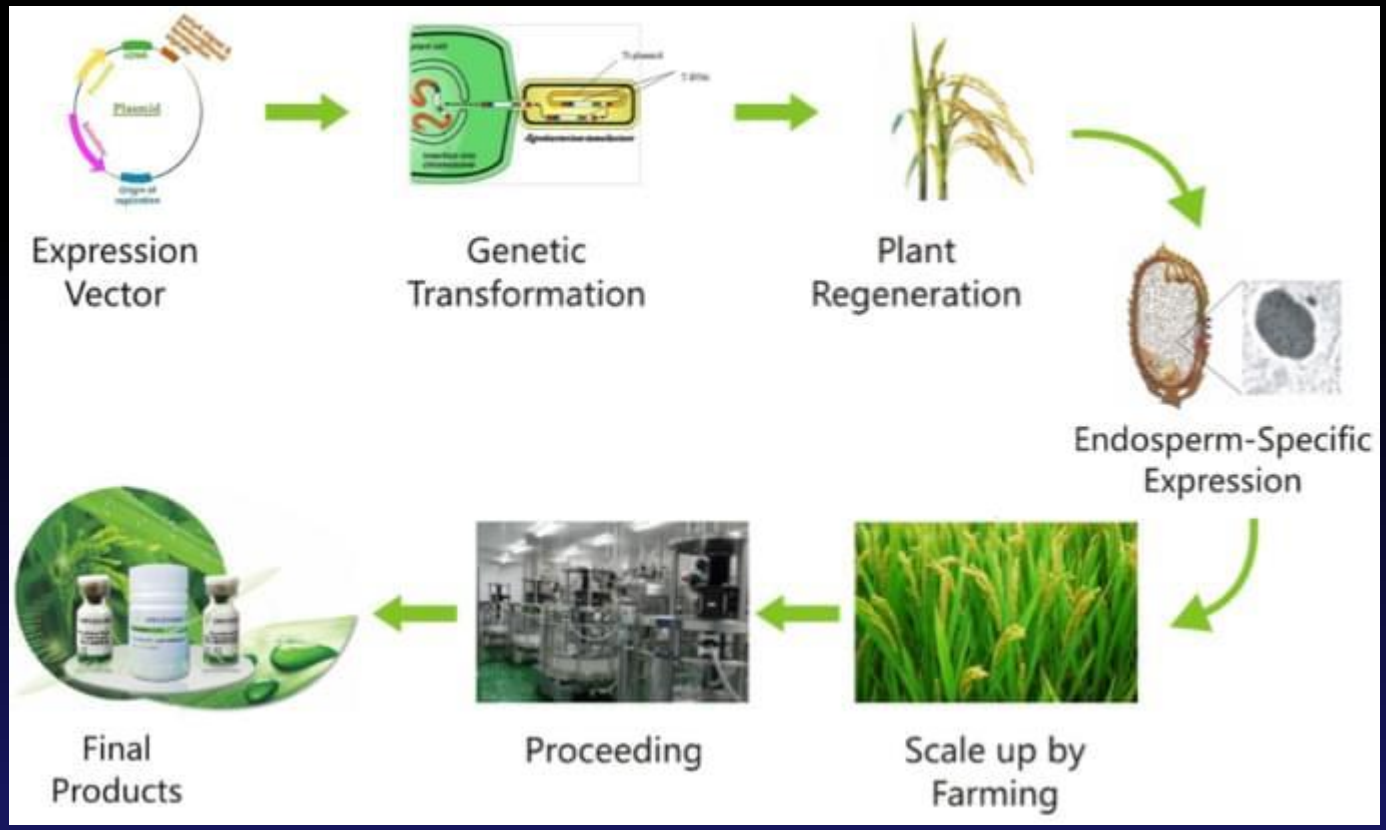


Schematic representation of the full virus vector strategy and of some examples of the deconstructed virus strategy. CP, coat protein; GOI, gene of interest; MP, movement protein; P, promoter; Pol, RNA-dependent RNA polymerase; RS, recombination site; T, terminator.

Source: Gleba Y, et al.,*Opin Plant Biol* **7**, 182-8.

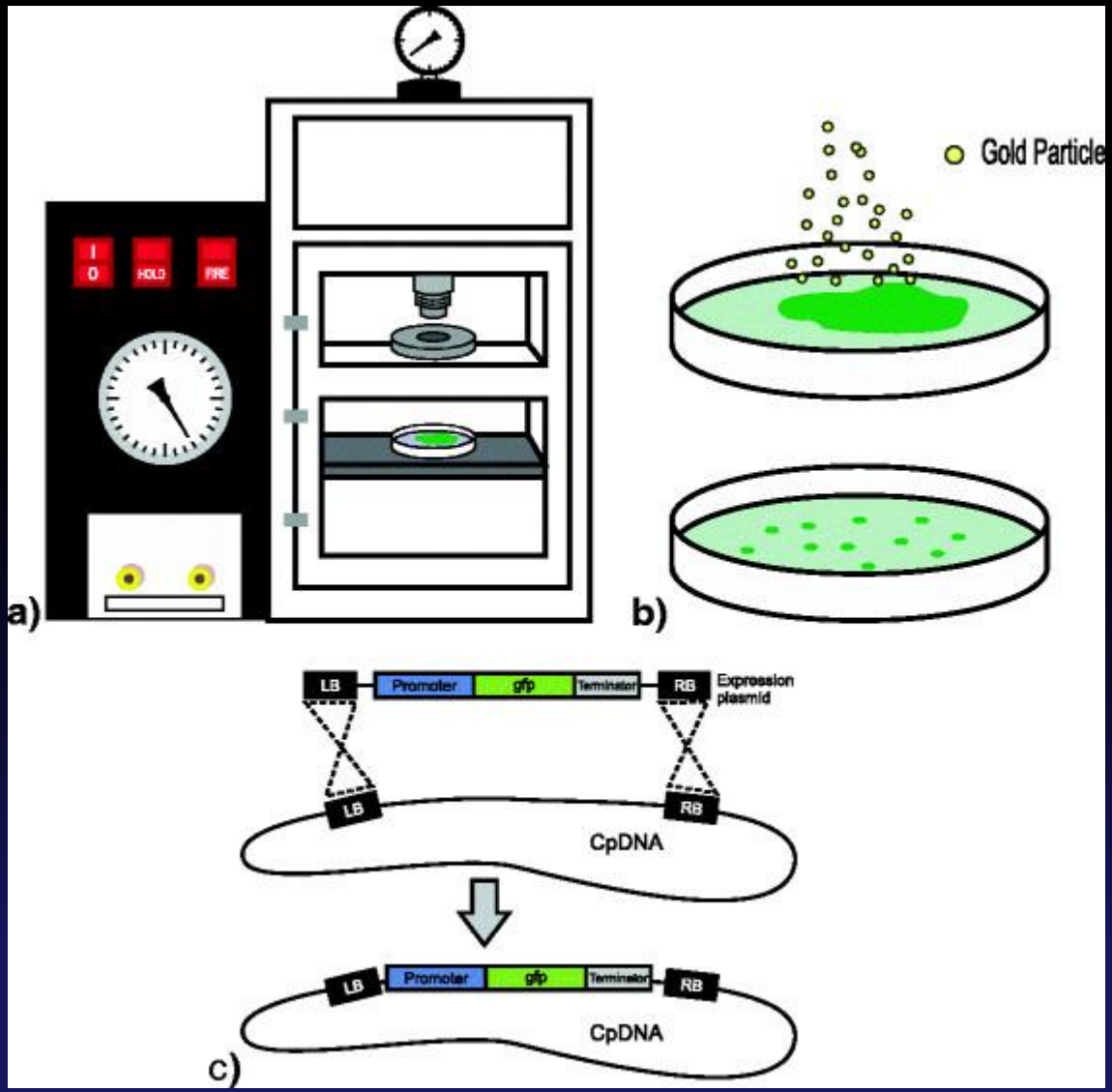
Seed-based expression

Seed-based expression



https://www.profacgen.com/Animal-free-Expression.htm

Chloroplast protein expression



three of the most import are: a) a product that is free of human pathogens and more quality-consistent; b) larger quantities can be obtained in a reduced or confined area and; c) low production cost when organisms are grown in economic media.

Moss cells to photosynthesize in culture significantly reduces the cost of culture nutrients

- ✓ Tumour-directed monoclonal antibodies with enhanced antibody-dependent cytotoxicity (ADCC),
- ✓ Vascular endothelial growth factor (VEGF), complement factor H (FH)
- ✓ Keratinocyte growth factor (FGF7/KGF) epidermal growth factor (EGF) Hepatocyte growth factor (HGF)
- ✓ Asialo-erythropoietin (asialo-EPO AEPO)
- ✓ Alpha-galactosidase (aGal) and beta-glucocerebrosidase (GBA)

Reski et al. 2015. *Plant biotechnology journal* **13**, 1191-8.

Summary & Drawbacks

- Expression level of transgenes in plants (epigenetics)
- Transformation capacities and trans-generational levels of expression
- Edible vaccines may trigger immune tolerance
- Most of the ingested protein will be degraded by digestive processes
- PMF is relatively new, microbial and animal cell expression systems have been used for over 30 years, and industry has developed standard and high-throughput purification protocols.
- Factors, such as plant phenolic compounds, plant pathogens, secondary metabolites, pesticides, and fertilizers, increase the difficulty of purifying a PMF product at an industrial level. Field crop-based PMF platforms, such as maize or rice, have pollen contamination issues which raise biosafety concerns as the pollen may contaminate non-transgenic crops that are part of normal agricultural production
- Subcutaneous injections of plant-derived proteins could induce an immunogenic response to plant-specific glycans

Literature and Further reading

- Researcher Engineers Protein-Rich Algae as Meat, Soy Substitute

  https://www.youtube.com/watch?v=2QvyBVFbaKk

- Yan N, Fan C, Chen Y, Hu Z, 2016. The Potential for Microalgae as Bioreactors to Produce Pharmaceuticals. *Int J Mol Sci* **17**, 962.
- Gleba Y, Marillonnet S, Klimyuk V, 2004. Engineering viral expression vectors for plants: the 'full virus' and the 'deconstructed virus' strategies. *Curr Opin Plant Biol* **7**, 182-8.
- Boothe J, Nykiforuk C, Shen Y, *et al.*, 2010. Seed-based expression systems for plant molecular farming. *Plant Biotechnol J* **8**, 588-606.
- Reski R, Parsons J, Decker EL, 2015. Moss-made pharmaceuticals: from bench to bedside. *Plant biotechnology journal* **13**, 1191-8.