PLNT2530 (2024)
Unit 10c
Applications of Plant Biotechnology in Agriculture

Novel Proteins

Plant Biotechnology
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Chapters 5-10

Production of Novel Protein Products - eg. Hirudin

Hirudin - a blood anticoagulant (thrombin inhibitor)

Small protein found in leech salivary gland

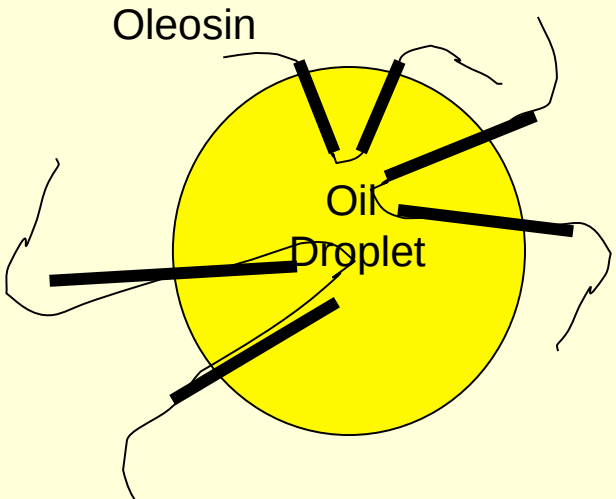
- Why plants?
  - Mammalian cell culture systems are expensive/not scaleable
  - Bacterial system (no s-s, no glycosylation or post-translational modification)
  - Seeds offer a way of producing large amounts of protein
  - Very cheap
  - Protein is concentrated
  - Can be stored for long periods (dry)
  - Seed have low hydrolytic activity

Technical challenges

- Seeds have high levels of complex storage proteins
  - Require means of purification of transgene product from other proteins

Potential solution:

- use a class of storage protein (oleosins) as a carrier
- Oleosins -small abundant proteins embedded in the phospholipid monolayer of oil bodies
- 8-12% of total rapeseed protein



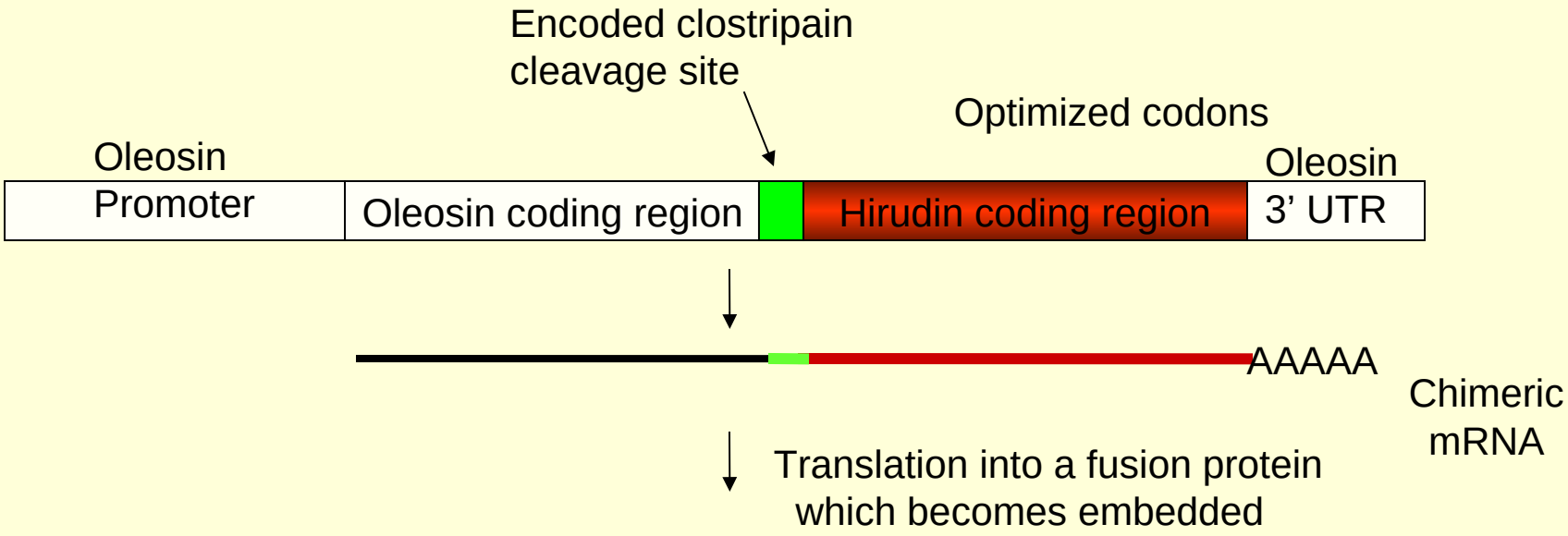
Oleosin protein from Arabidopsis

- MADTHRVDRT DRHFQFQSPY EGGRGQGQYE GDRGYGGGGY
  KSMMPESGPS STQVLSLLIG VPVVGSLLAL AGLLLAGSVI
  GLMVALPLFL LFSPVIVPAA LTIGLAMTGF LASGMFGLTG
  LSSISWVMNY LRGTRRTVPE QLEYAKRRMA DAVGYAGQKG
  KEMGQHVQNK AQDVKQYDIS KPHDTTTKGH ETQGRTTAA 199

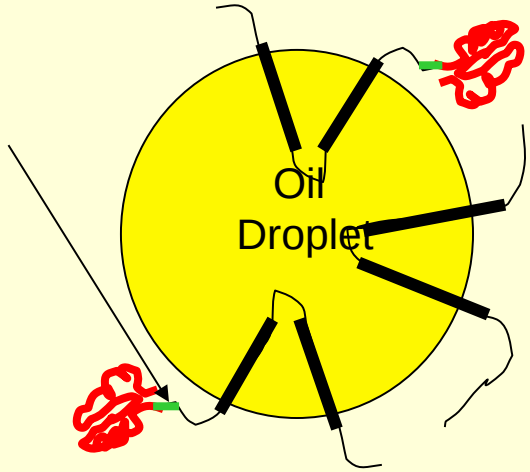
- Underlined region is transmembrane spanning regions with hydrophobic region consisting of non-polar amino acids

- Hydrophilic N and C terminal regions with acidic (D, E) and basic (K, R, H) amino acids are in aqueous phase ie. outside of oil bodies

Expression in oilseed



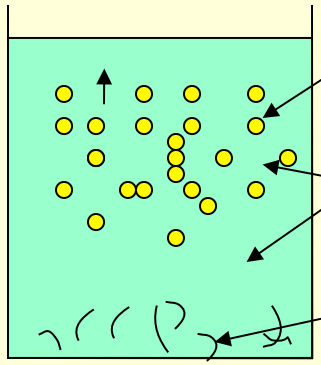
(IleGluGlyArg)
clostripain cleavage site



Purification of fusion protein

Transgenic rapeseed with oleosin-hirudin chimeric gene

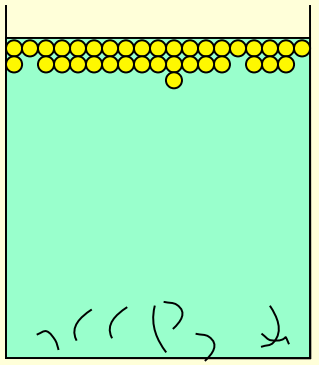
Homogenize in aqueous solution



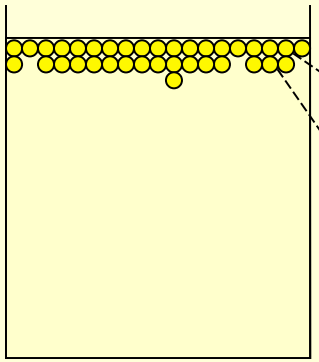
Oil bodies with associated oleosin-hirudin fusion protein

Soluble proteins

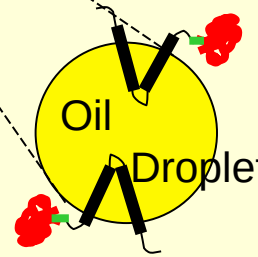
Insoluble debris



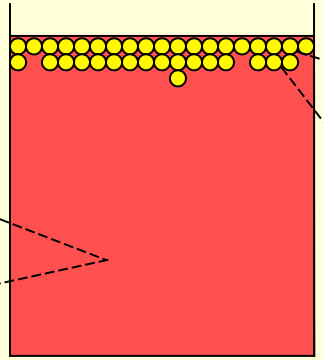
Collect oil layer and wash several times to remove soluble proteins and insoluble material

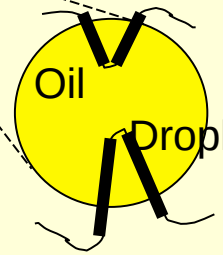


Digest with clostripain



Hirudin released Into aqueous phase





Patented technology

Production of Ebola virus antisera by transient expression in tobacco

Neutralizing antibodies bind to virus particles, preventing the virus from binding to cellular receptors. Neutralizing antibodies from patients recovering from a virus have been show to be good theraputic agents. In medicine, a number of diseases can be treated by harvesting immunoglobulins (gamma globulins) from individuals who have recovered from a viral infection to provide some level of immunity to patients with infectious diseases.

By expressing recombinant antibody genes in transgenic plants, the plant can produce large quantities of neutralizing antibodies which can be harvested from plant tissue.

1. Determine amino acid sequences of antibodies to Ebola Sudan strain (US military)
2. Create monoclonal antibody line for the target sequence
3. Clone synthetic antibody genes into T-DNA vector (PlantForm and Univ. of Guelph.
4. Transform *N. benthianum* tobacco plants with *Agrobacterium*. Cells in the transformed sectors will express the immunoglobulin proteins.
5. After 1 week, protein is extracted from T_0 plants. Use of T_0 plants speeds up the process of responding to new strains of the virus.

You don't need a large amount of immunoglobulins to have an effective treatment.

http://www.marsdd.com/news-and-insights/ontario-biotech-plantform-tobacco-plants-ebola-vaccine/

Infiltration of leaf tissue with Agrobacterium tumefaciens

Using a hypodermic without a needle, draw bacterial suspension into the hypodermic.

Press the mouth of the hypo against the leaf, supporting the leaf from behind with a gloved finger.

Depress the plunger to force bacterial suspension into intercellular spaces.


Plant Form
Our Manufacturing Process
From seedling to high-value biopharmaceutical in eight weeks
Plant Nicotiana benthaminia seeds
Plants grow, generating biomass
6 weeks
Grow agrobacterium containing genetic sequence for target drug
3 days
Infiltrate plants with agrobacterium
Plants grow, producing drug in each cell
1 week
Extract drug
1 day
Purify drug
4 days
Fill vials
1 day
Package product
1 day

Medicago (medicago.com) vaccine for Covid19 - Virus-Like Particles produced through transient expression in *Nicotiana benthamiana*

T-DNA plasmid carrying lentivirus coat protein genes, and gene for Covid19 spike protein.

Infiltrate Agro. into intercellular spaces of leaves and allow to incubate.

Plant produces virus-like particles, which are purfied from leaves.

February 20222 - Was approved for production in Canada

March 2022 - WHO rejects Medicago vaccine because the tobacco company, Philip Morris, owned 1/3 of Medicago. WHO policy forbids business with tobacco companies.

Parent company of Medicago shuts down production of the vaccine.