Gene Delivery

- Programme: B.Sc (H) Botany
- Course Title: Plant Biotechnology
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Gene delivery

- The gene delivery methods can be divided into two categories, viral and non-viral.
- Virus mediated gene delivery utilizes ability of a virus to inject its DNA inside a host cell. A gene that is intended for delivery is packaged into a viral particle.
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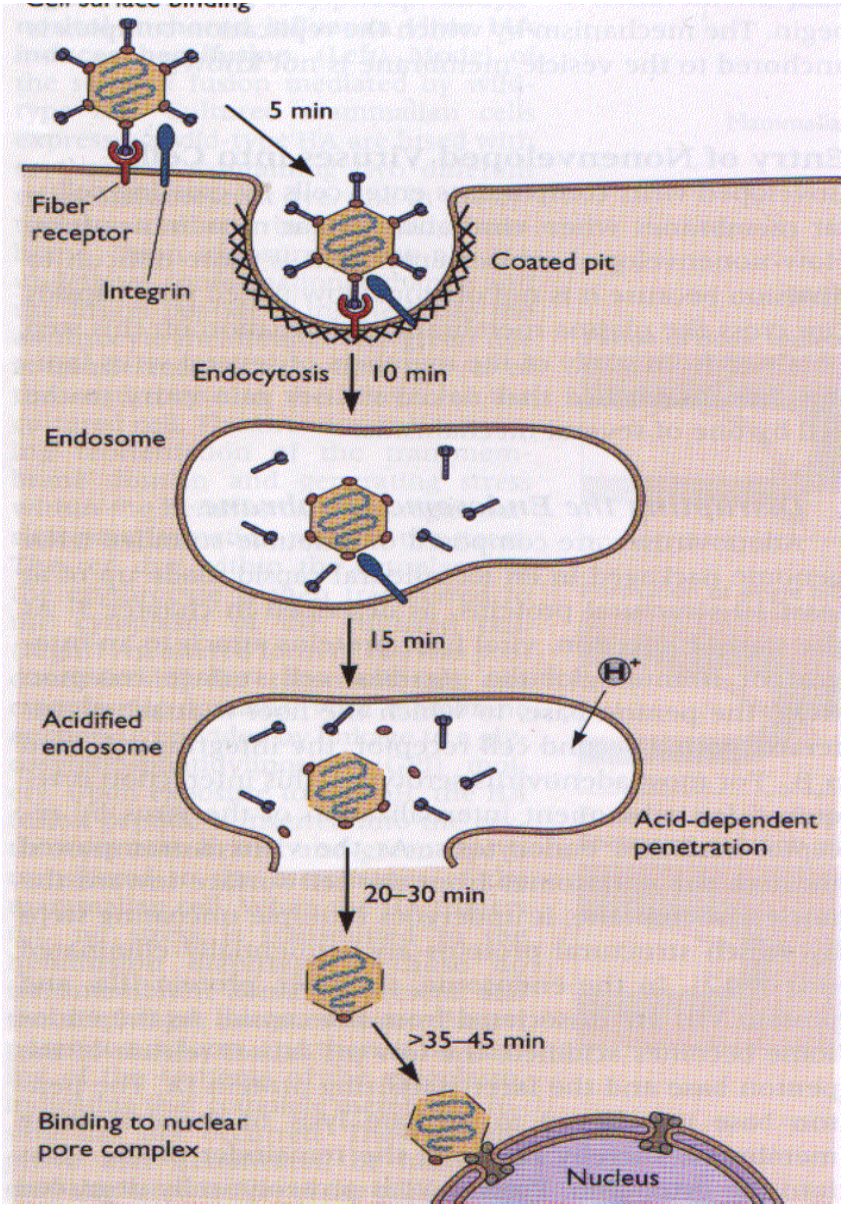
Virus mediated

- Plant viruses are used as vectors for transferring gene to plant genome for modification of the host genome
- The efficient infection machinery and comprehensive genome structure makes viral genomes excellent choice to be used as vectors.
- Autonomously replicating virus-based vectors provide alternative means to deliver genes to plant cells.

Viral vectors



Compared to naked DNA, virus particles provide a relatively efficient means of transporting DNA into cells, for expression in the nucleus as recombinant genes *(example = adenovirus)*.

[figure from Flint *et al.* Principles of Virology, ASM Press, 2000]

Viral vectors

- Both DNA viruses (*Bean yellow dwarf virus, Wheat dwarf virus* and *Cabbage leaf curl virus*) and
- RNA virus (*Tobacco rattle virus*) have demonstrated efficient gene targeting frequencies in model plants (*Nicotiana benthamiana*) and crops (potato, tomato, rice, and wheat).

https://www.frontiersin.org/articles/10.3389/fpls.2017.00539/full

Example

- In recent years, *Barley stripe mosaic virus* **BSMV** has become a popular vector for virus-induced gene silencing (**VIGS**) in barley and wheat.
- In **VIGS**, a short fragment of a transcribed sequence of a plant gene is inserted into a cloned virus genome, and the recombinant virus is then inoculated onto test plants

http://www.plantphysiol.org/content/160/2/582#F1

- The introduced virus multiplies and spreads from the site of infection into newly developing regions of the plant and triggers posttranscriptional gene silencing.

Non-viral methods include

i)physical methods such as microinjection, gene gun, electroporation, by using liposomes and

ii) agrobacterium based method

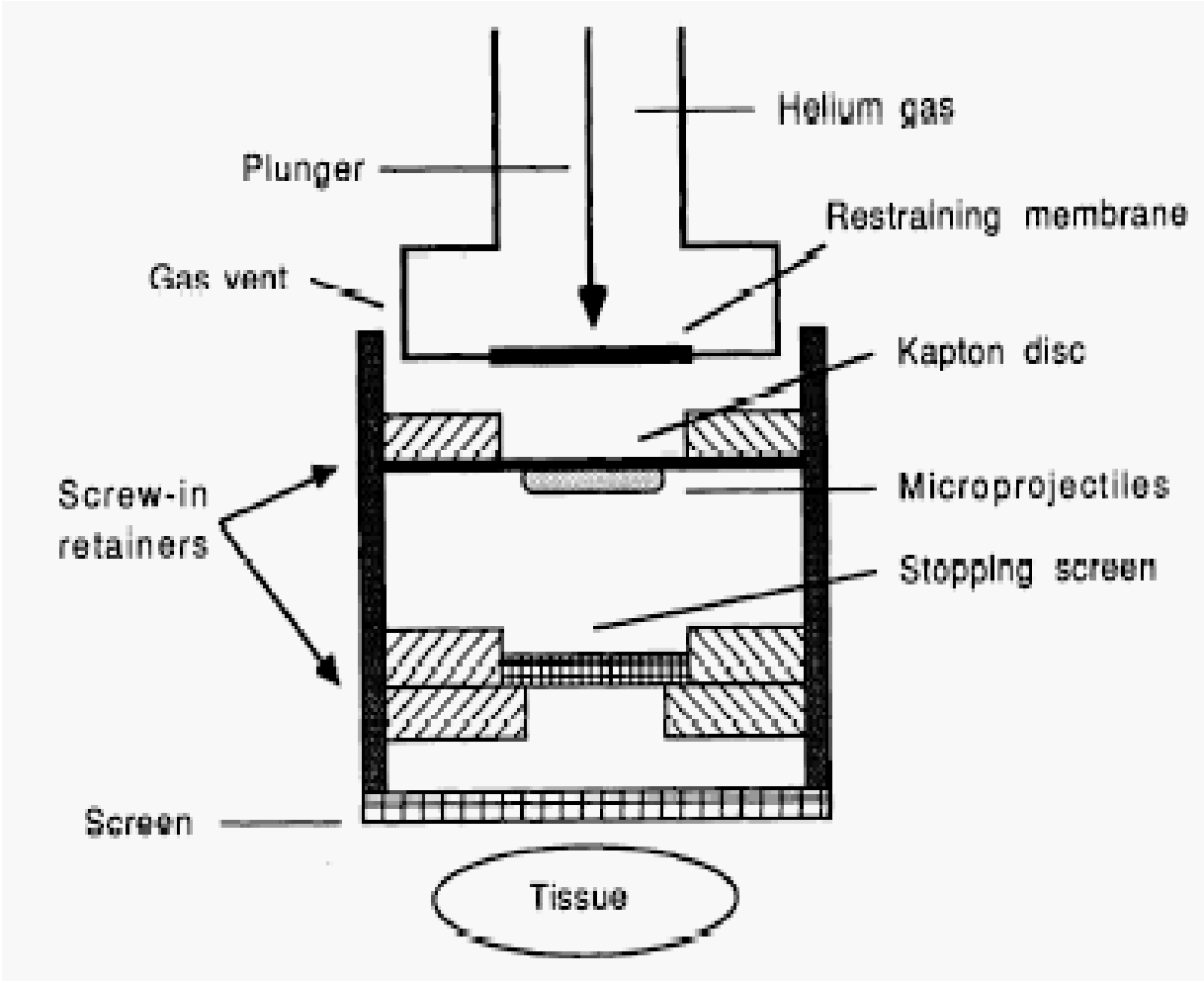
Physical methods

- These are often called as direct methods as they can place the gene in the receiving host directly without the aid of any other organism.

Microprojectile bombardment / Gene gun

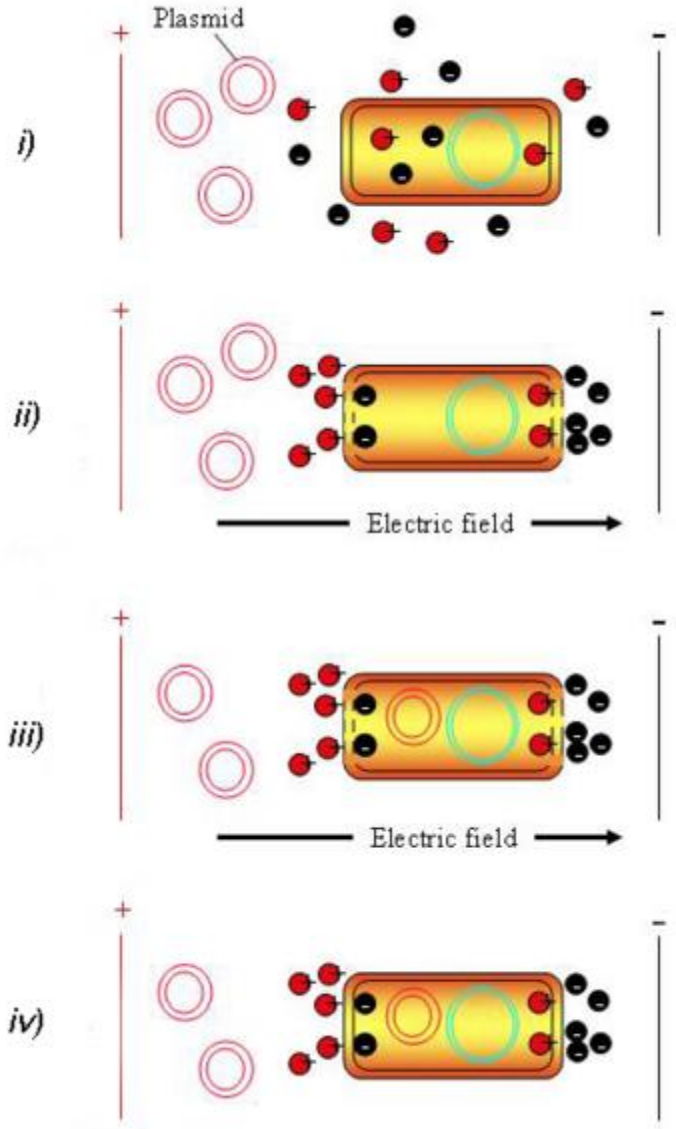
- The gene gun is part of a method called the biolistic (also known as bioballistic) method, and under certain conditions, DNA (or RNA) become “sticky,” adhering to biologically inert particles such as metal atoms (usually tungsten or gold). By accelerating this DNA-particle complex in a partial vacuum and placing the target tissue within the acceleration path, DNA is effectively introduced.

- Uncoated metal particles could also be shot through a solution containing DNA surrounding the cell thus picking up the genetic material and proceeding into the living cell. A perforated plate stops the shell cartridge but allows the slivers of metal to pass through and into the living cells on the other side.


Helium gas
Plunger
Restraining membrane
Gas vent
Kapton disc
Screw-in
retainers
Microprojectiles
Stopping screen
Screen
Tissue

- The cells that take up the desired DNA, identified through the use of a marker gene (in plants the use of GUS is most common), are then cultured to replicate the gene and possibly cloned. The biolistic method is most useful for inserting genes into plant cells such as pesticide or herbicide resistance.
- Different methods have been used to accelerate the particles: these include pneumatic devices; instruments utilizing a mechanical impulse or macroprojectile; centripetal, magnetic or electrostatic forces; spray or vaccination guns; and apparatus based on acceleration by shock wave, such as electric discharge

- This technique is most suitable for those plants which hardly regenerate and do not show sufficient response to gene transfer through *Agrobacterium* for example, rice, wheat, corn and banana



Electroporation

Electroporation
is a technique in which
an electrical field is applied to cells in order to increase the permeability of the cell membrane, allowing chemicals, drugs, or DNA to be introduced into the cell

i) When the electric field is applied, the ions move according to their charge.

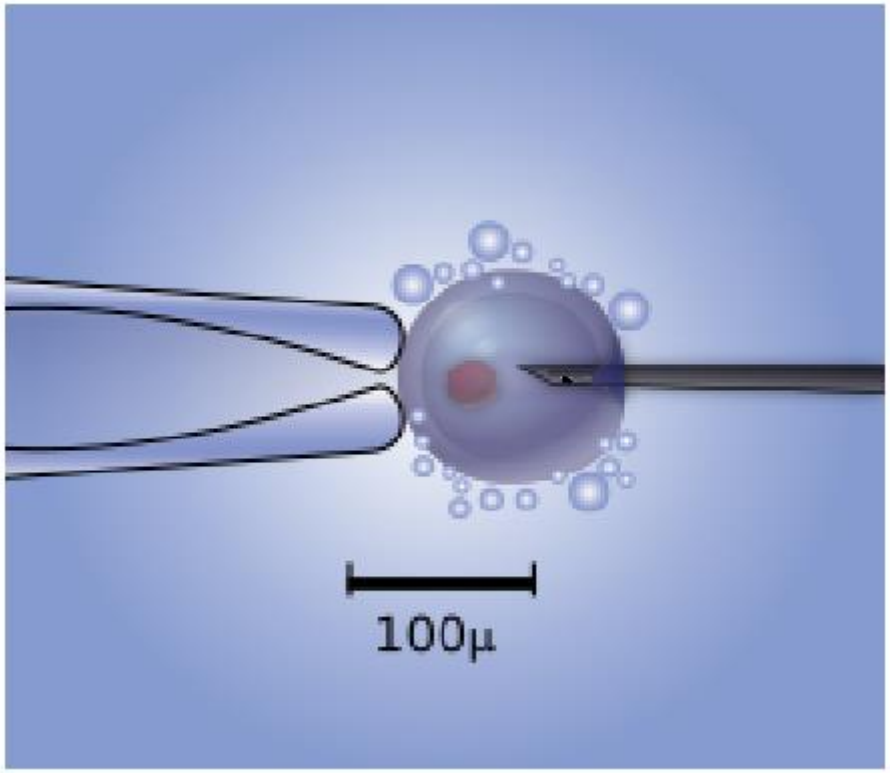
ii) Pathways are formed across the cell membrane allowing DNA to enter.

iii) When the electric field is deactivated, the membrane heals.

Microinjection

- **Microinjection** - In case of microinjection, the DNA solution is injected directly inside the cell using capillary glass micropipettes with the help of micromanipulators of a microinjection assembly. It IS easier to use protoplasts than cells since cell wall interferes with the process of microinjection.

  The protoplasts are usually immobilized in agarose or on glass coated with poly lysine or by holding them under suction by a micropipette. The process of microinjection is technically demanding and time-consuming; a maximum of 40-50 protoplasts can be microinjected in one hour.



https://en.wikipedia.org/wiki/Microinjection

- When cells or protoplasts are used as targets in the technique of microinjection, glass micropipettes each with 0.5-10µm diameter tip are used for transfer of macromolecules into the cytoplasm or the nucleus of a recipient cell or protoplast. The recipient cells are immobilized on a solid support (cover slip or slide, etc.) or artificially' bound to a substrate or held by a pipette under suction (as done in animal systems).

- It is necessary to introduce the DNA into the nucleus or the cytoplasm of the cell for high transformation rates. Therefore, it is the most successful with densely cytoplasmic, nonvacuolated embryonic cells.

  Successful transformation by microinjection of cells/protoplasts has been achieved in tobacco, alfalfa, Brassica sp. etc., the transformation frequencies ranging between 14 and 66%. But this approach has been disappointing with cereals.

Resources

- Sung, Y. K., & Kim, S. W. (2019). Recent advances in the development of gene delivery systems. *Biomaterials research*, *23*(1), 8.
- Free web resources