Protoplast fusion for culture development

Protoplasts

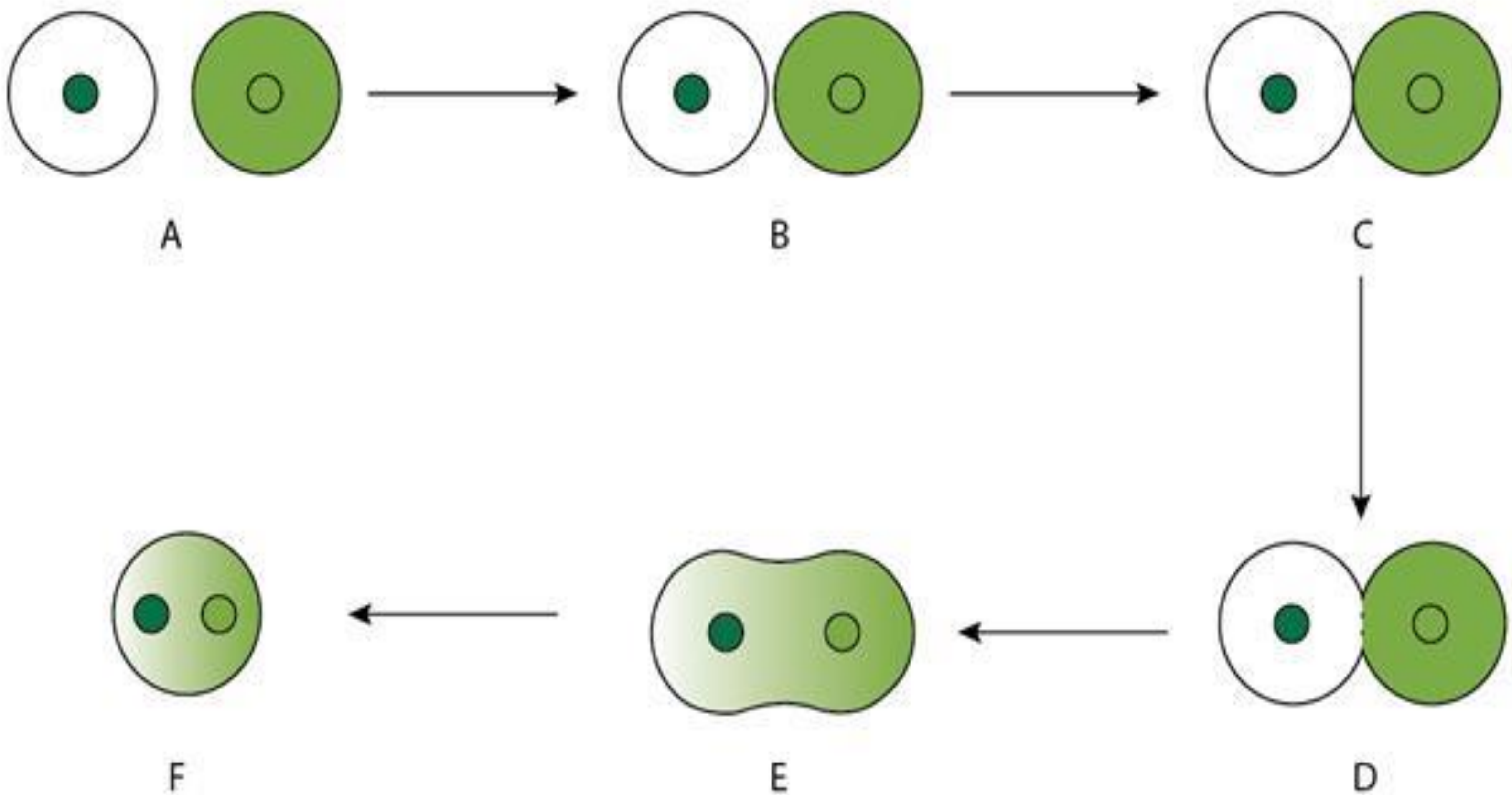
Protoplasts are the cells with **cell walls removed.**

So **cytoplasmic membrane** is the **outermost layer** in such cells.

Protoplasts can be obtained by specific **lytic enzymes** to remove cell wall.

Protoplast fusion

- Protoplast fusion is a **physical phenomenon**.
- During fusion, two or more protoplasts **come in contact and adhere** with one another **either spontaneously** or **in presence of fusion inducing agents.**


A
B
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- By protoplast fusion it is possible to transfer **some useful genes** from **one species (or even genus) to another.**

- These include genes for disease resistance, nitrogen fixation, rapid growth rate, more product formation rate, protein quality, frost hardiness, drought resistance, herbicide resistance, heat and cold resistance.

- Protoplast fusion is an important tool in **strain improvement,** for bringing **genetic recombinations** and developing **hybrid strains** esp. in filamentous fungi.

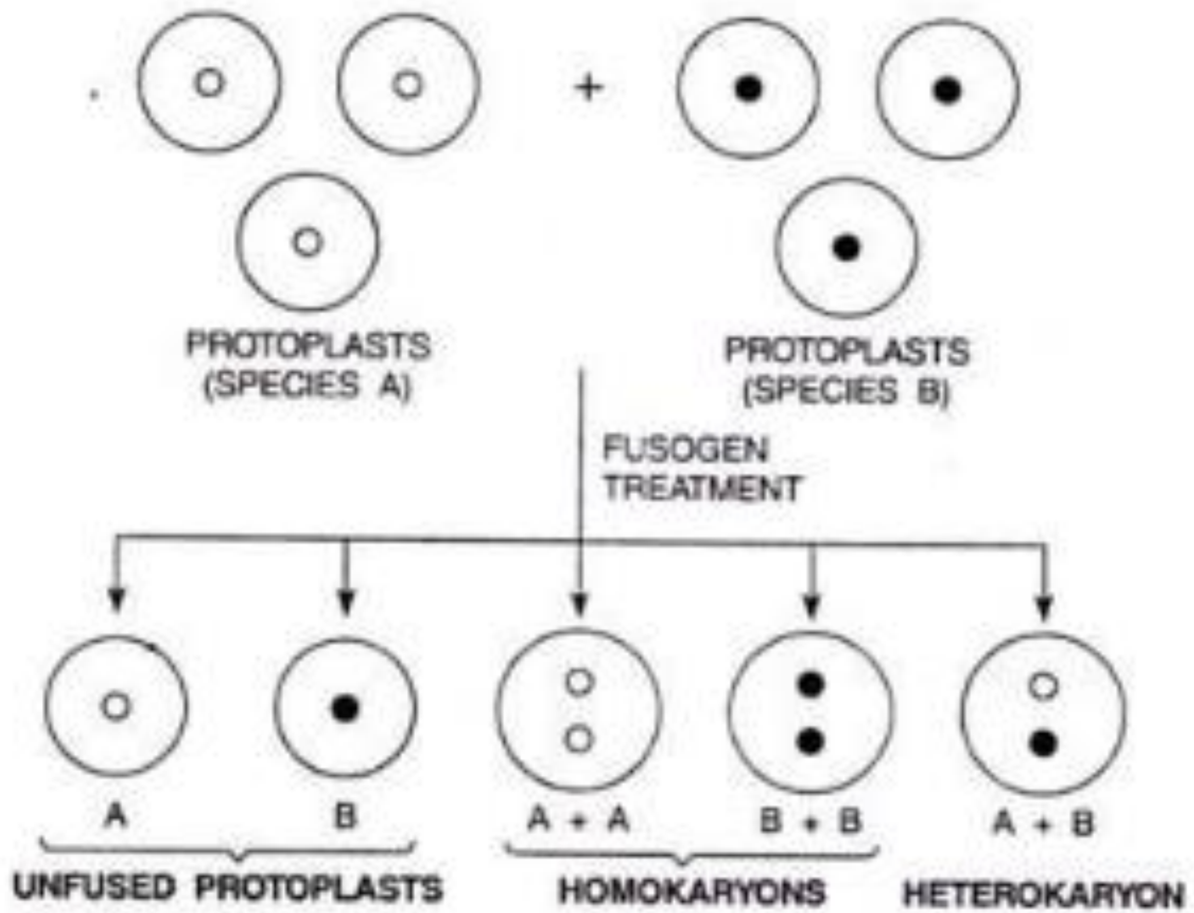
- Protoplast fusion has been used to **combine genes from different organisms** to create strains with desired properties, e.g. industrial properties.

- It will continue to be an **exciting** area of research in modern biotechnology.

- In **future**, this technique will be one of the **most frequently used** research tools for tissue culture scientists, molecular biologists, biochemical engineers and biotechnologists.

Hybrid protoplasts

- In protoplast technology, two **genetically different protoplasts** isolated from the somatic cells are experimentally fused to obtain **parasexual hybrid protoplasts**.
- The hybrid protoplast contain **heteroplasoic cytoplasm** (more than one source of mitochondrial DNA) and **two fused parent nuclei**.
- Protoplast fusion may be used to produce **interspecific** or **even intergeneric hybrids.**
- Protoplast fusion becomes an important tool of gene manipulation because it **breaks down the barriers to genetic exchange** imposed by conventional mating systems.



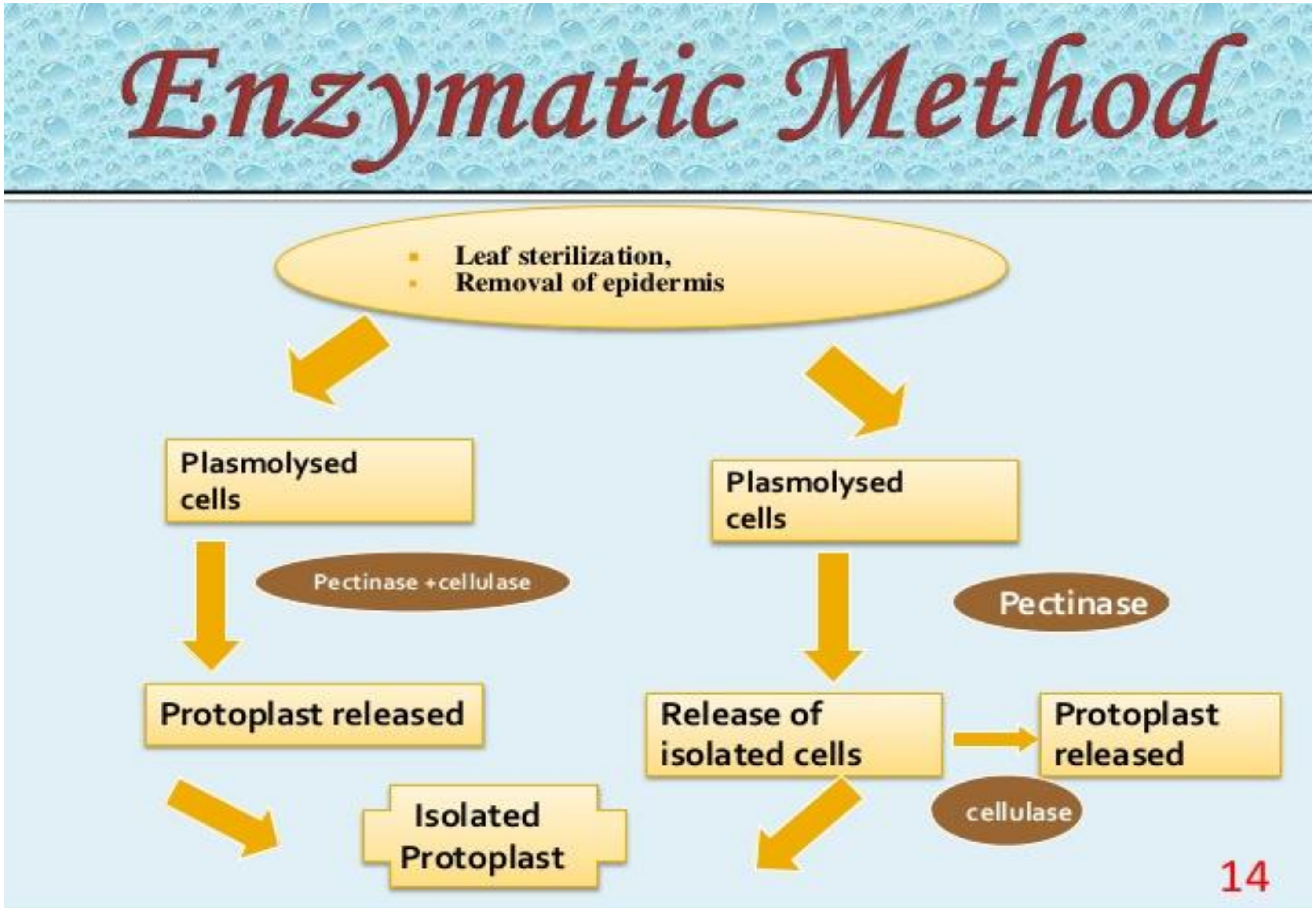
+
PROTOPLASTS
(SPECIES A)
PROTOPLASTS
(SPECIES B)
FUSOGEN
TREATMENT
A
B
A + A
B + B
A + B
UNFUSED PROTOPLASTS
HOMOKARYONS
HETEROKARYON

Enzymes used for breaking of cell walls

- For protoplast fusion it is important that the cell wall of plant cells or microorganisms is **degraded**.

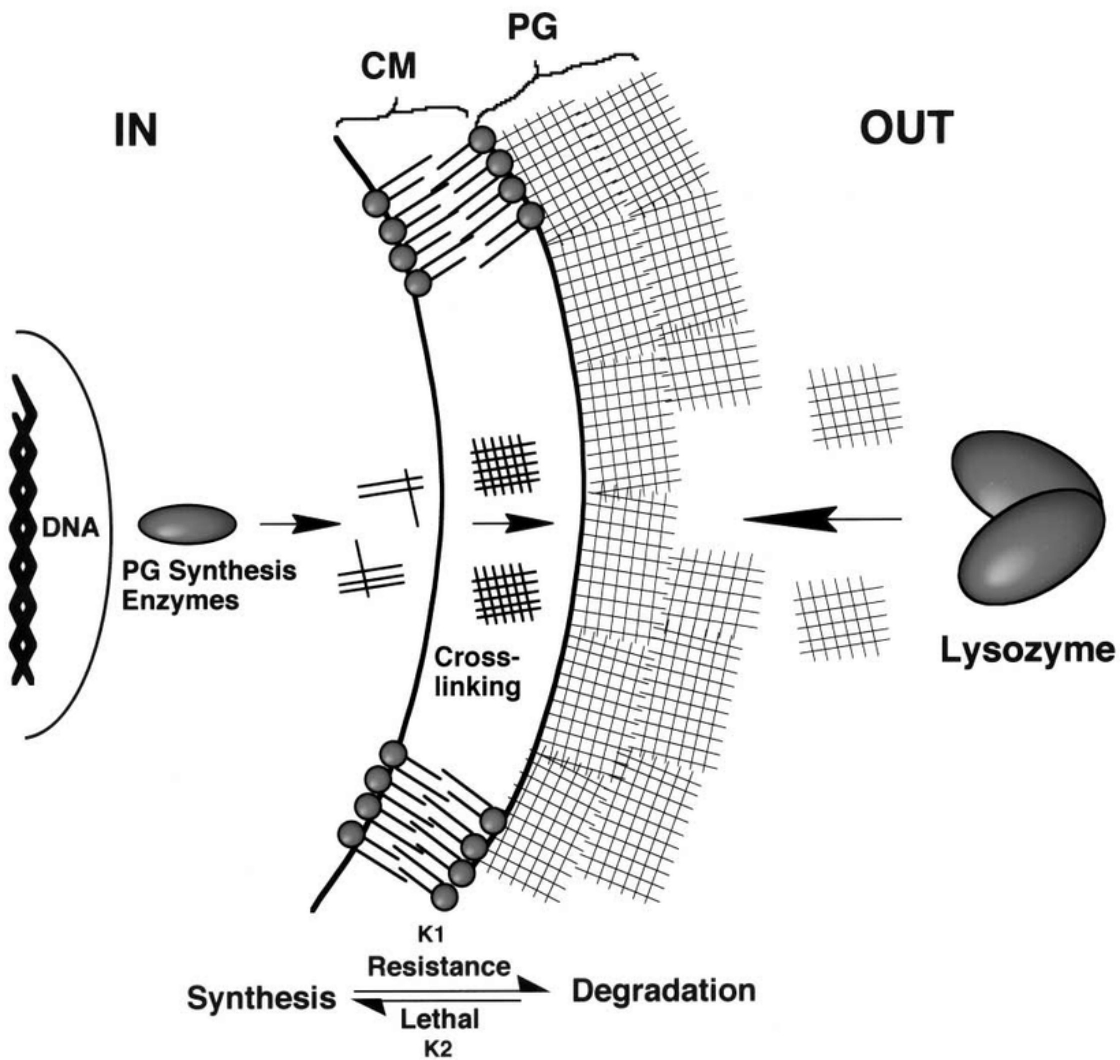
- So various **enzymes** are used for this process.

- **Cellulase, pectinase** and **macerozyme** are examples of enzymes acting on **plant cell wall**.



Protoplast release in plant cells

Bacterial cell walls are degraded by the action of **lysozyme**.



- **Fungal cell wall** is degraded by **Novozyme -234** which includes **glucanase** and **chitinase.**

- ***Streptomyces* cell wall** is degraded by action of **lysozyme** and **achromopeptidase**.

Methods of protoplast fusion

Protoplast fusion can be broadly classified into **two** categories:

1. **Spontaneous fusion**

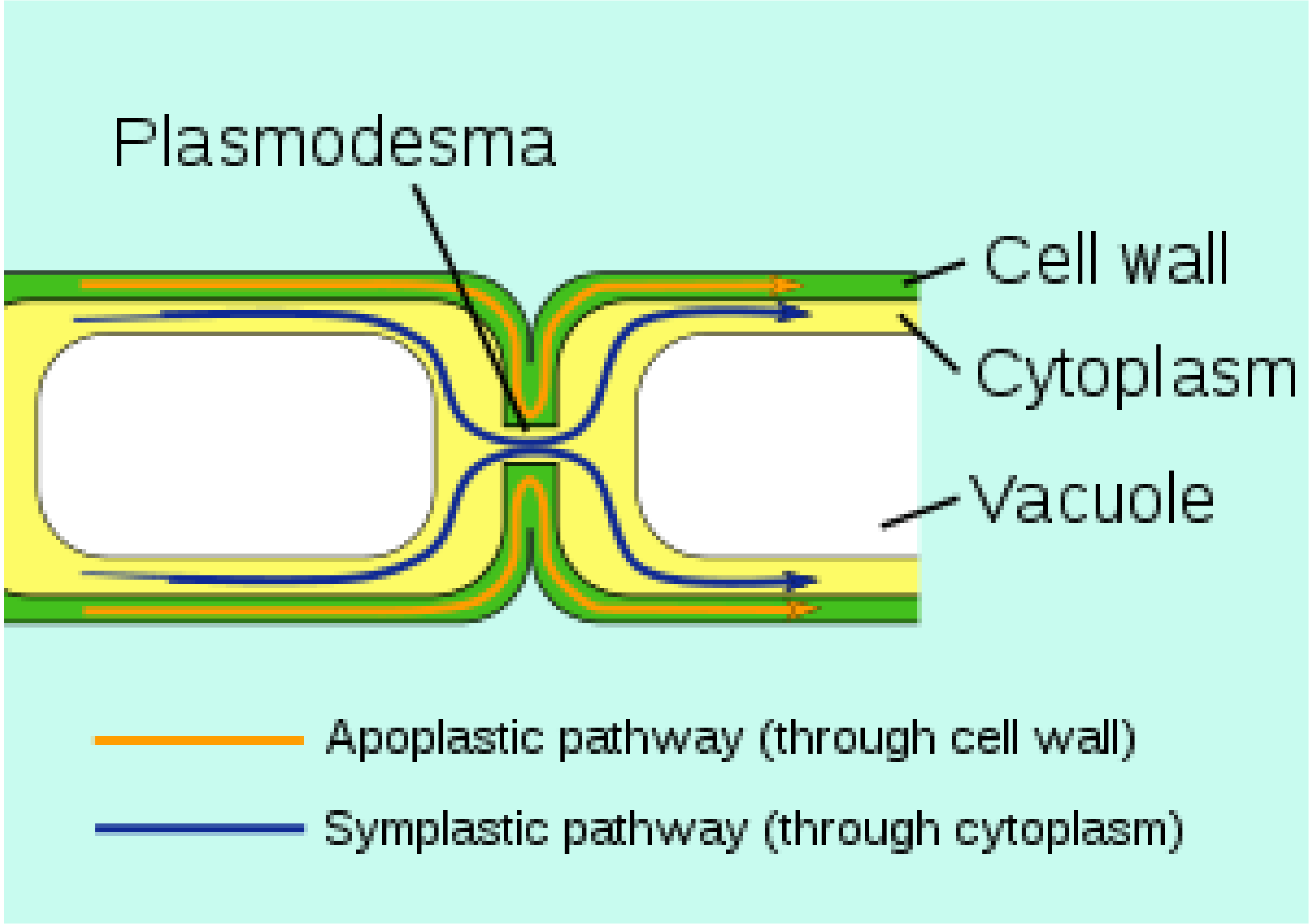
2. **Induced fusion**

Spontaneous fusion:

- Protoplast during isolation often **fuse spontaneously** and this phenomenon is called **spontaneous fusion**.

- In plants, during the enzyme treatment, protoplast from adjoining cells fuse through their **plasmodesmata (plasmodesma)** to form **multinucleate protoplasts.**

Plasmodesmata is a narrow thread of cytoplasm that passes through the cell walls of adjacent plant cells and allows communication between them.



Plasmodesma
Cell wall
Cytoplasm
Vacuole
Apoplastic pathway (through cell wall)
Symplastic pathway (through cytoplasm)

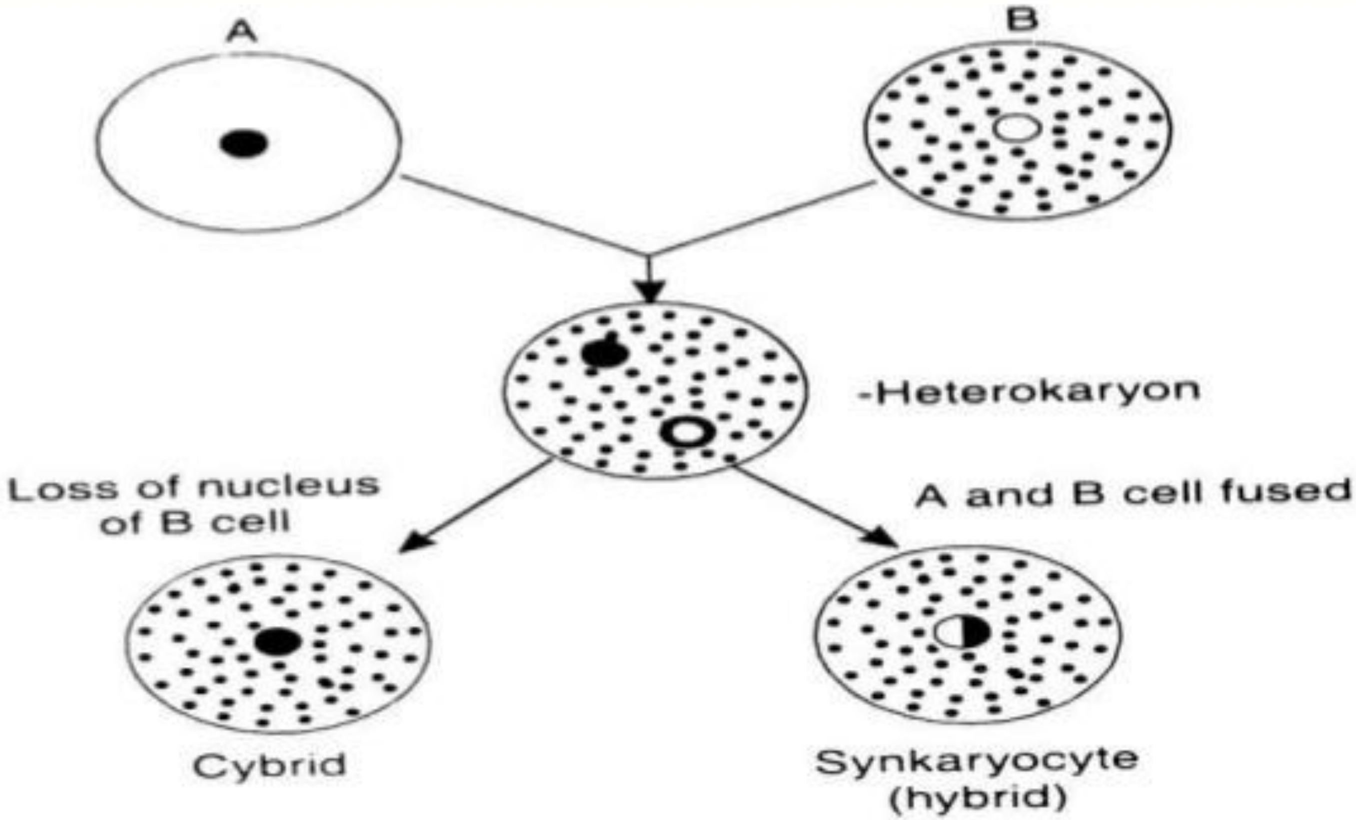
By spontaneous fusion we may obtain:

1. **Cybrid:**

   Nucleus of one of the fusing cells is totally lost and remaining nucleus represent only one of the fusing cells.

2. **Hybrid (synkaryocyte):**

   The nuclei of two fusing cells fuse with each other to form a single nucleus.

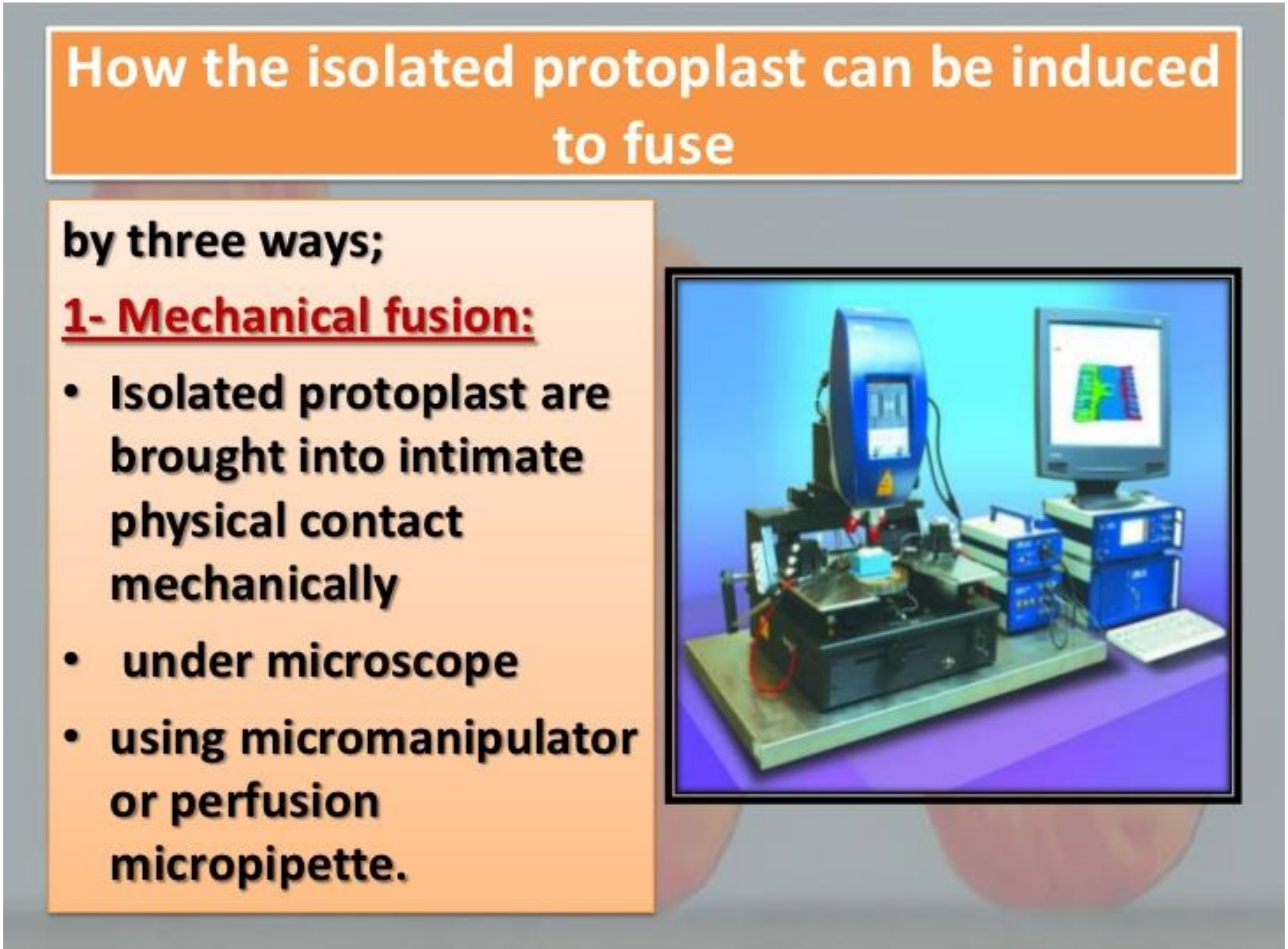

A
B
-Heterokaryon
Loss of nucleus
of B cell
A and B cell fused
Cybrid
Synkaryocyte
(hybrid)

Induced fusion:

The isolated protoplast can be induced to fuse by **three** ways:

1. **Mechanical fusion**
2. **Chemofusion**
3. **Electrofusion**

1. Mechanical fusion:



2. Chemofusion:

- Several chemicals have been used to induce protoplast fusion such as **sodium nitrate, polyethylene glycol,** and **Calcium ions (Ca++).**

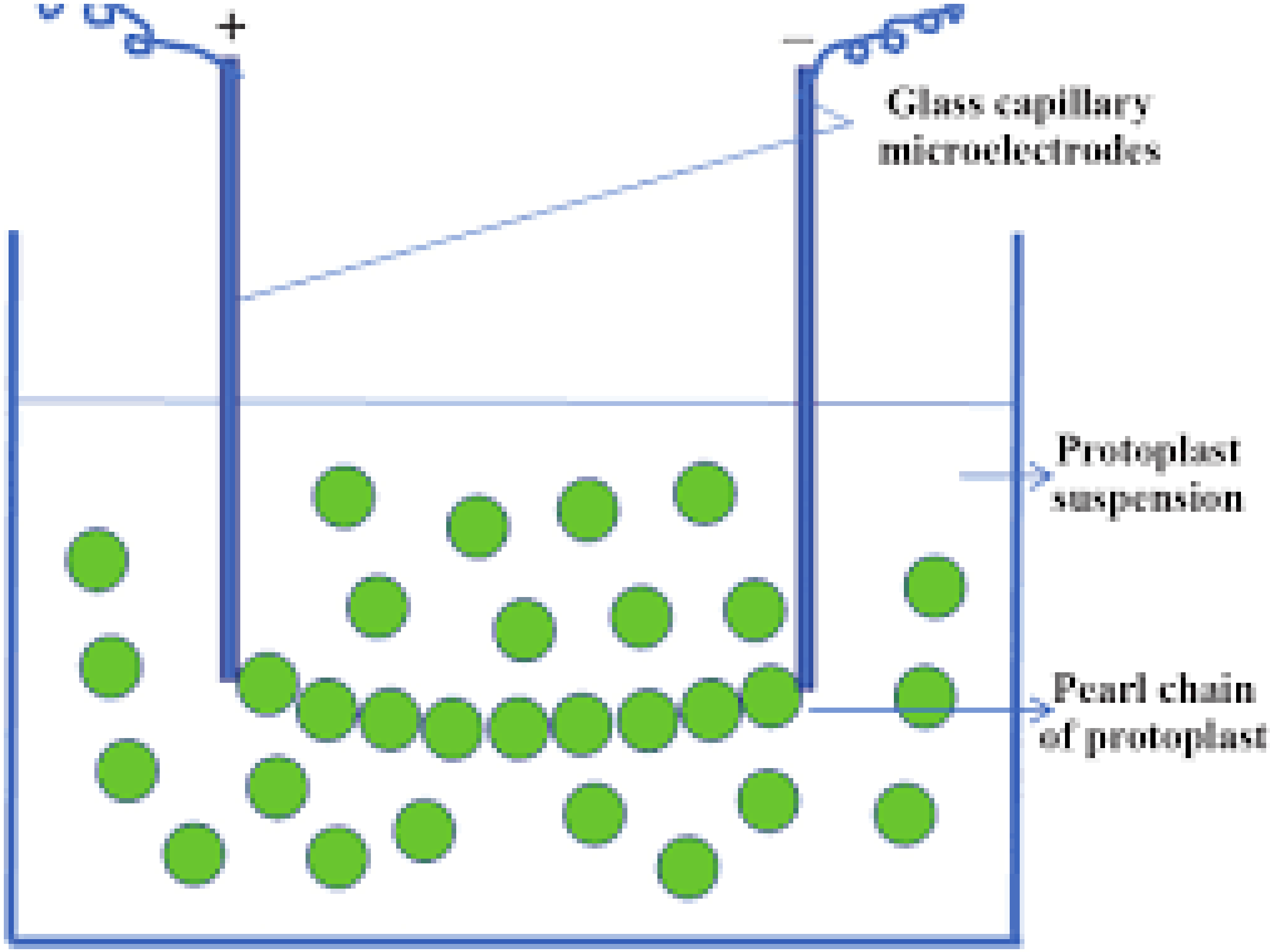
- **Chemical fusogens** cause the isolated protoplast to **adhere to each other** and leads to **tight agglutination** followed by fusion of protoplast.

- Chemofusion is a **non specific, inexpensive technique** which can cause **massive fusion product**.

- But it can be **cytotoxic and non-selective** and have **less fusion frequency.**

3. Electrofusion:

- Recently, **mild electric stimulation** is being used to fuse protoplast.
- In this, two **glass capillary microelectrode** are placed in contact with the protoplasts (in solution).
- An electric field of low strength (10Kvm-1) gives rise to **dielectrophoretic dipole generation** within the protoplast suspension.
- This leads to **pearl chain arrangement** of protoplasts.


+
–
Glass capillary microelectrodes
Protoplast suspension
Pearl chain of protoplast

- **Subsequent** application of **high strength of electric fields** (100 kvm-1r) for some **microseconds** results in electric breakdown of membrane and subsequent fusion.

- Initial experiment was on ***Penicillium*** protoplasts, and diaelectrophoresis yielded viable fusion products.

- **Pulse electric field** for diaelectrophoresis has also been used.

- Giant protoplasts of ***Pleurotus cornucopiae*** were fused using the glass microelectrode fusion technique.

- To induce fusion, **Ca++** are necessary.
- **Polyethylene glycol 400 (PEG)** not only promotes fusion but also increase the adhesion of protoplasts.
- Electrofusion is easy to control with fusion frequency **up to 100% and it gives reproducibility.**
- It is also **less cytotoxic**.
- But equipment is **sophisticated and expensive**.

Mechanism of protoplast fusion

- The mechanism of protoplast fusion is **not fully known**. Several explanations have been put forward to understand the mechanism of protoplast fusion.

- When the protoplasts are brought into close proximity, **changes induced in electrostatic potential of the membrane** results in fusion.

- After the fusion, the **membranes stabilizes** and the surface potential returns to its former state.

- It is also suggested that when the protoplasts are closely adhered, the external fusogens **cause disturbance in the intramembranous proteins and glycoproteins**.

- This increases **membrane fluidity** and creates a region where **lipid molecules intermix** with each other, allowing merger of adjacent membranes.

- The **negative charge** carried by protoplast is mainly due to **intramembranous phosphate groups**.

- The addition of **Ca++** causes **reduction in the zeta potential of plasma membrane** and under this situation protoplasts are fused.

- **Protoplast fusion** takes place when the **molecular distance between the protoplasts is 10A (1 nanometer) or less.**

Zeta potential is the potential difference existing between the surface of a solid particle immersed in a conducting liquid (e.g. water) and the bulk of the liquid.

Protoplast fusion in fungi

- Production and regeneration of protoplasts is a useful technique for **fungal transformations**.
- Commercial preparation of enzymes contain mixture of products to digest fungal cell wall.
- Protoplast fusion frequency in fungi is **0.2 -2%.**

Biotechnological applications of protoplast fusion

- The **direct bioconversion of cellulosic materials to ethanol** by the intergeneric fusants between *Trichoderma reesei* and *Saccharomyces cerevesie* appears to be one of the best technique as an **alternative approach for ethanol production**.

- Also this process is helpful in the **production of a complete set of cellulases** by the protoplast fusion of *T. reesei* and *A. niger* (one produces endo- and exoglucanase and other produces ß-glucosidase).

- Intraspecific protoplast fusion with polyethylene glycol 1000 in *Streptomyces griseoflavus* has been used to increase the production of **desferrioxamine B chelator** that absorbs additional iron from the blood of **thalasemia** patients.

- **Electrically induced protoplast fusion** has been employed for hybrid construction in **ergosterol producing yeast strains**.

- Fusants of *Penicillium chrysogenum* and *Cephalosporium acremonium* have produced a **novel beta-lactam antibiotic**.

- Protoplast fusion in *Streptomyces avermitillis* showed improved properties in respect to **rifampicin resistance**.


Thank You!