HYBRIDOMA TECHNOLOGY

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HISTORY

- In 1975, these technology developed by **Georges J.F.Kohler and Cesar Milstein.**
- And in 1984, they shared a Nobel prize for this discovery.
- They make a hybrid cell that will make a numbers of monoclonal antibodies against antigen .

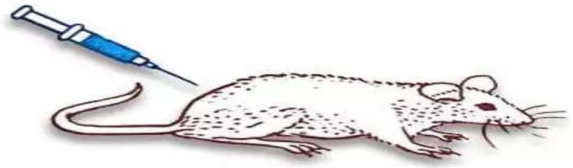
PRINCIPLE

- The hybrid cell has the capacity of antibody production derived from B-cells (spleen cell).
- At the same time it can divide continuously by the quality derived from myeloma cell.
- By combining the desired qualities of both the cells, the technology ensures large, antibody production of single specificity.
- Specific hybridomas(spleen cell and myeloma cell) obtain monoclonal antibodies in artificial media, this technology called as **HYBRIDOMA TECHNOLOGY.**

PROCEDURE

1. Immunization of specific animal which generate hybridoma cell with spleen cell.
2. Isolation of myeloma cells.
3. Fusion between spleen cell and myeloma cell.
4. Selection of HAT medium.
5. Isolation of hybridoma cell.
6. Screening of hybridoma cell.

1. IMMUNIZATION OF SPECIFIC ANIMAL



- An antigen immunized to an animal (like mice) via intravenously(directly to blood) by injection.
- Where in spleen it activate B-cell which produce plasma cell (spleen cell).
- Plasma cell to produce monoclonal antibodies
- Isolation of plasma cell from spleen of animal.

2. ISOLATION OF MYELOMA CELLS

- Myeloma cells are cancerous cells which is isolated from bone-marrow.
- Myeloma cells are generally **immortal** in nature (that which never dies) and has multiplication property.

3. FUSION OF SPLEEN CELL AND MYELOMA CELL

- It requires PEG (poly ethylene glycone) medium for fusion
- It can also done by electro fusion.
- Fusion between spleen cell and myeloma cell produced five different types of cells.
  - Fused plasma
  - Fused myeloma
  - **Hybridoma**
  - Unfused plasma
  - Unfused myeloma

4. SELECTION OF HAT MEDIUM

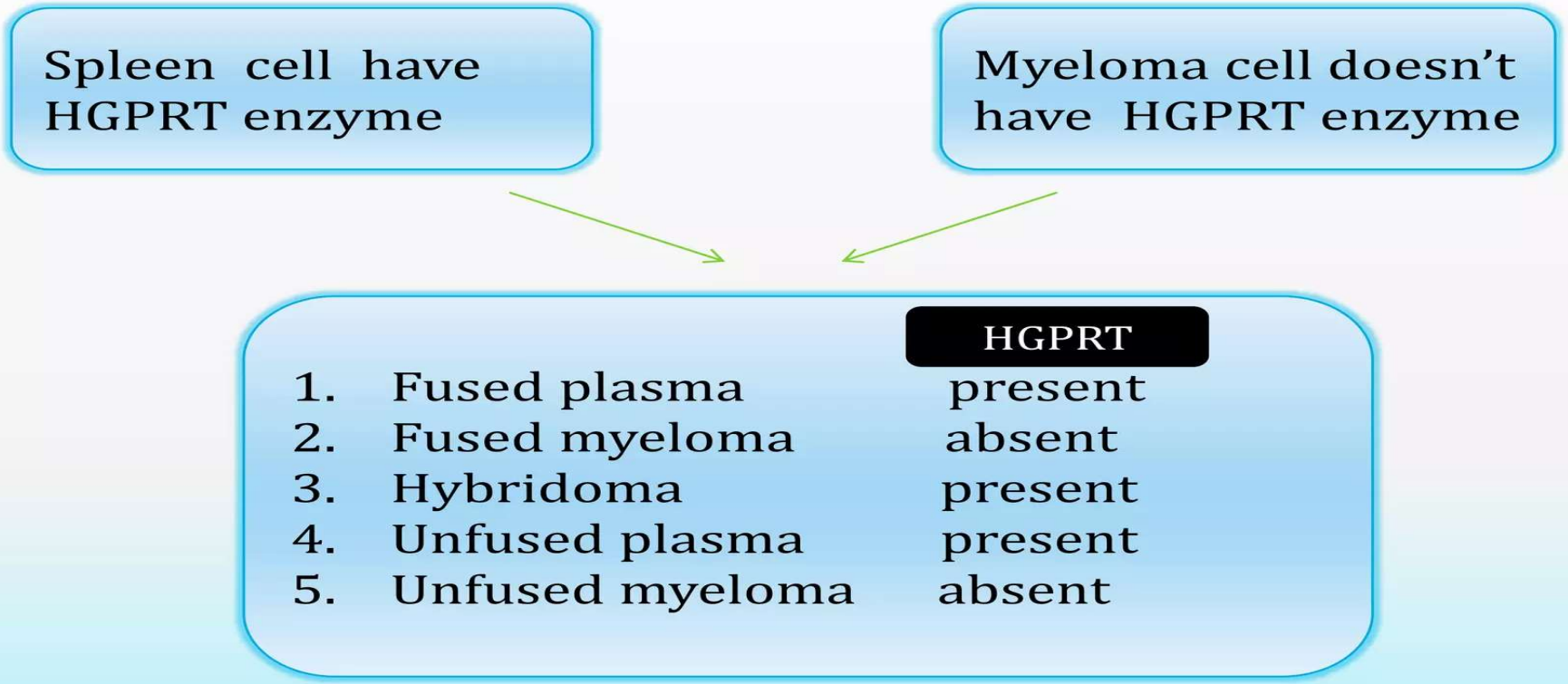
(Hypoxanthine, Aminopterin, Thymidine)

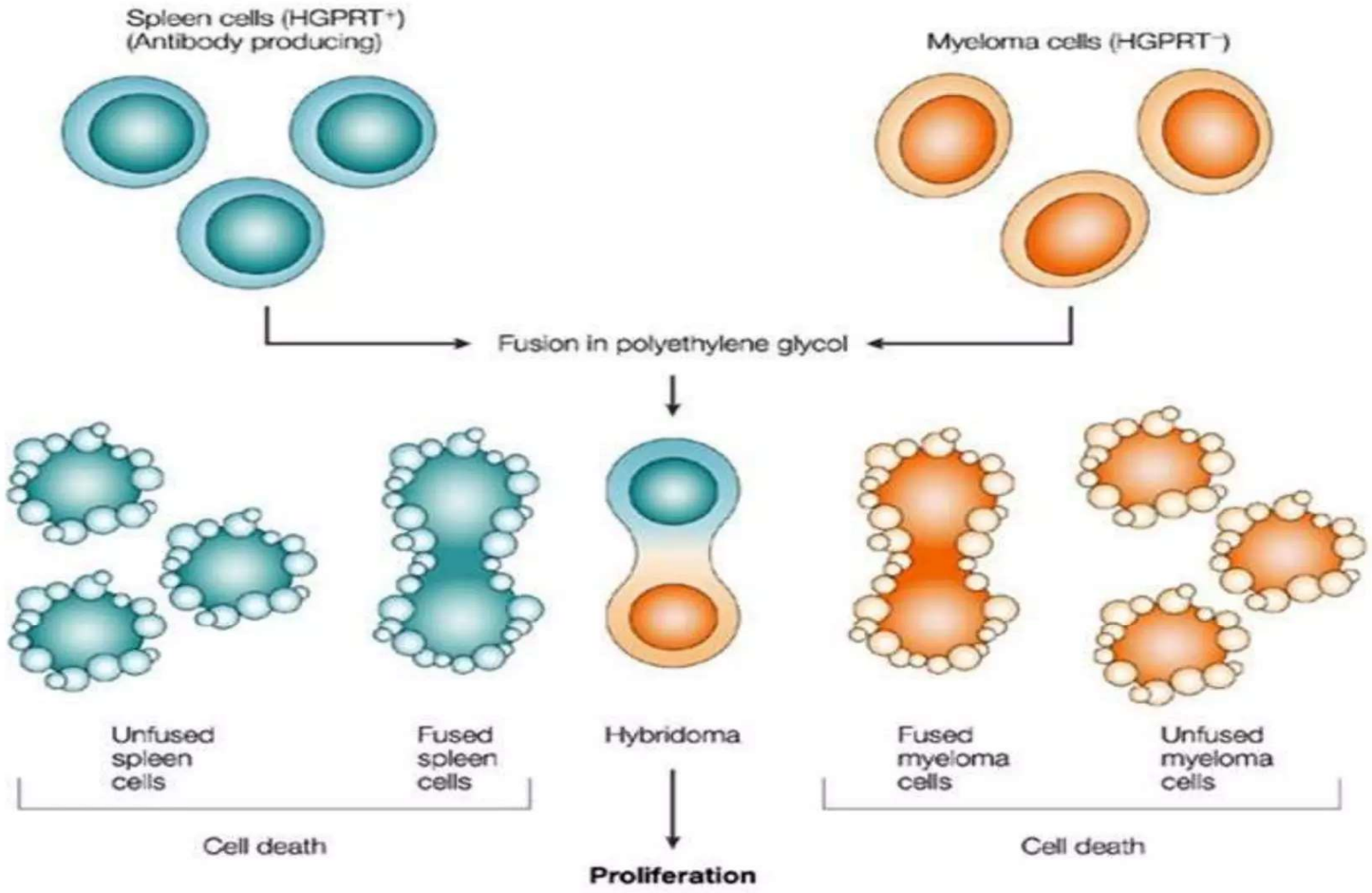
- Before multiplication of Anti-body, it has to synthesize new copy of DNA and for that it require synthesis of nucleotide.
- For synthesis of nucleotide mainly two pathways are there:

  1. Salvage pathway
  2. De-novo Synthesis
- In 1 , Salvage pathway it requires degraded part of old nucleotide to produce new nucleotide.
- In 2, De-novo synthesis it synthesized completely new nucleotide by small molecules (sugar, amino-acid).

- So in HAT medium, Cells not synthesized by De-novo synthesis due to presence of Aminopterin in HAT medium which blocks **Di-hydro follate enzyme** which is necessary for these synthesis.
- For synthesis in salvage pathway it must requires **HGPRT** enzyme (Hypoxanthine Guanine Phospho-Ribosyl Transferase).
- Where hypoxanthine and thymidine are used as precursors.

5. ISOLATION OF HYBRIDOMA CELLS

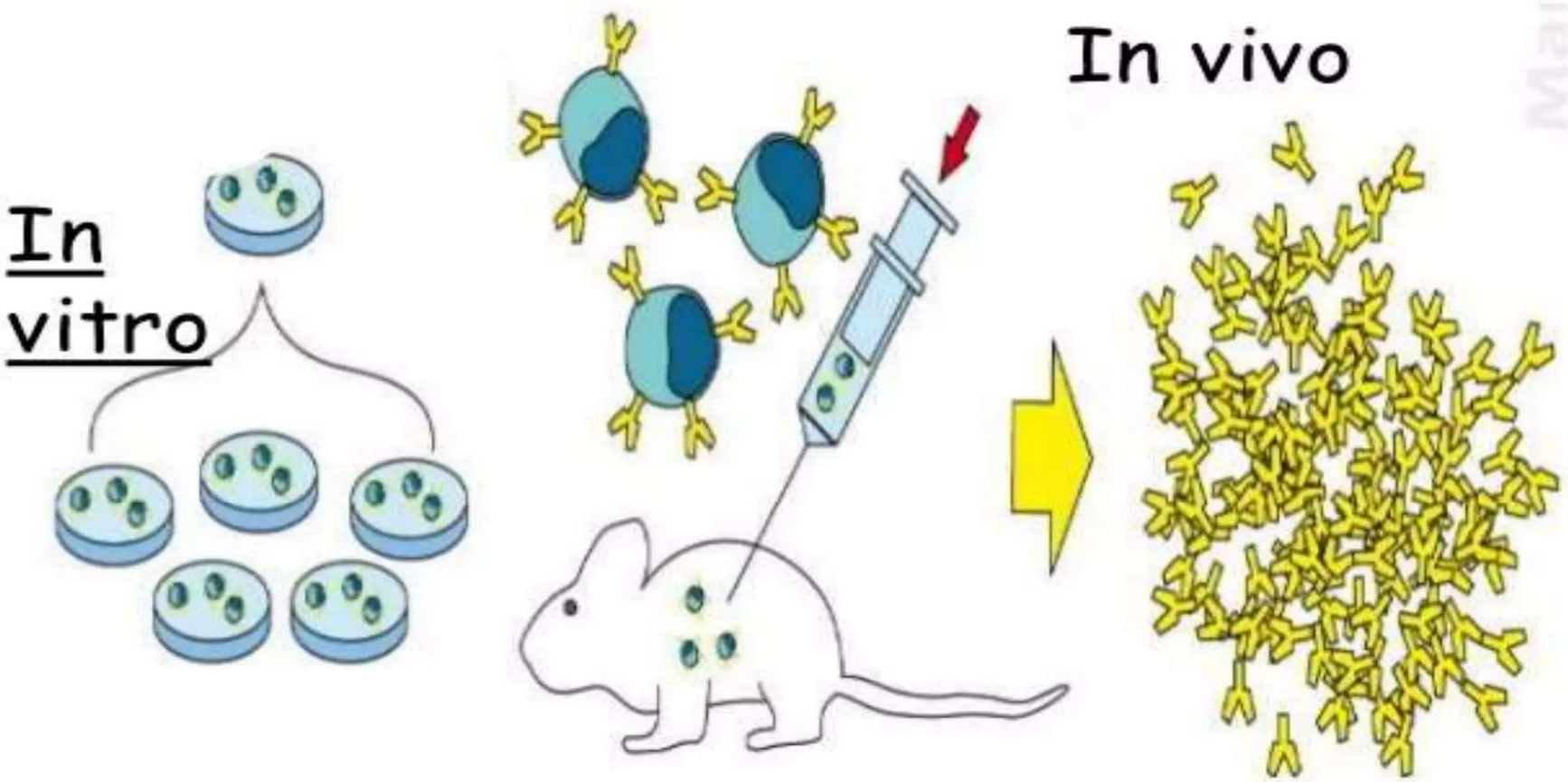



Spleen cells (HGPRT+)
(Antibody producing)
Myeloma cells (HGPRT−)
Fusion in polyethylene glycol
Unfused spleen cells
Fused spleen cells
Hybridoma
Fused myeloma cells
Unfused myeloma cells
Cell death
Proliferation
Cell death

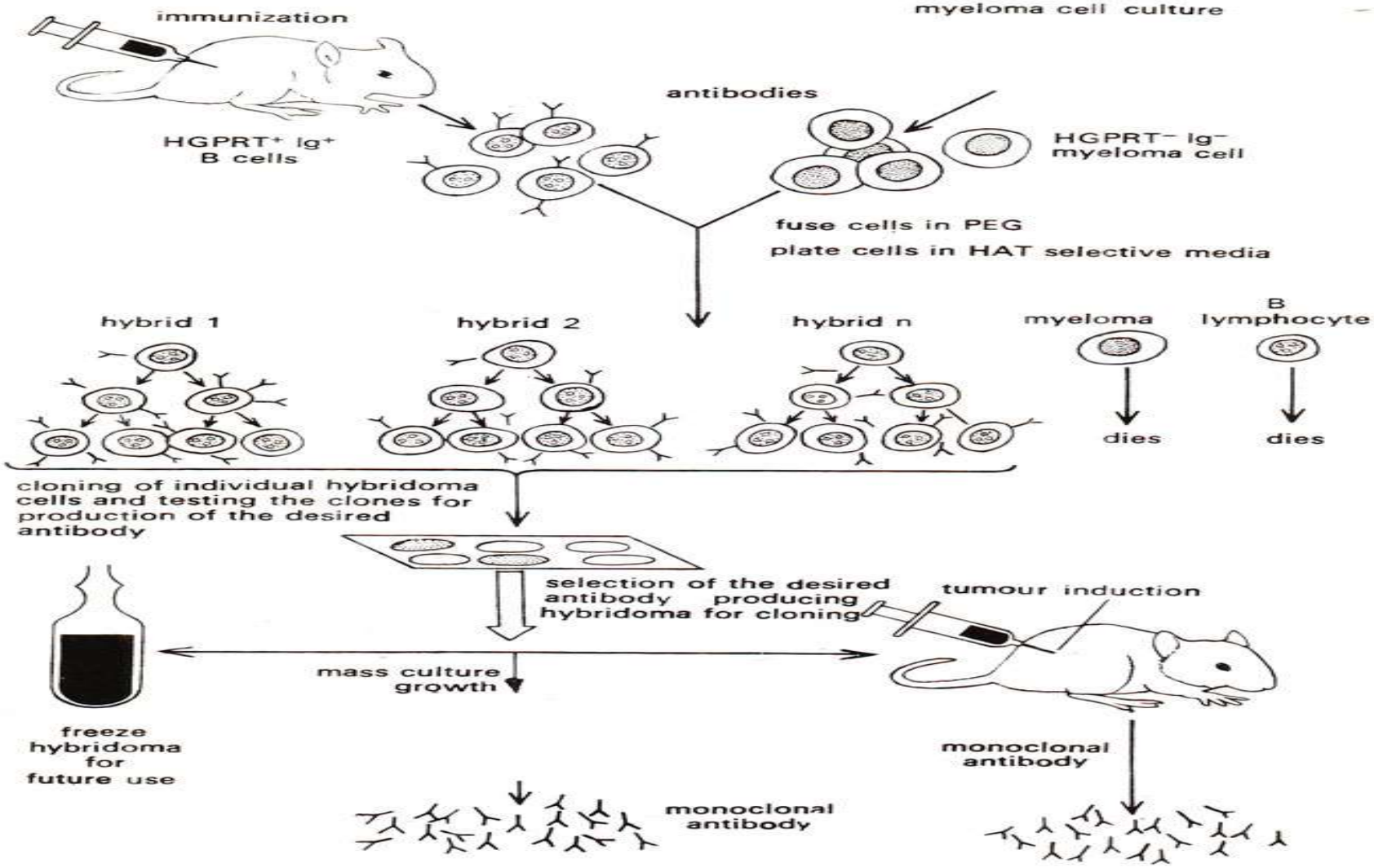
- Fused myeloma and unfused myeloma didn't have HGPRT enzyme so, can't survive in HAT medium.
- Fused plasma and unfused plasma have HGPRT enzyme but didn't have long-life.
- **Hybrid cell** has HGPRT enzyme from spleen cell as well as they have the ability to multiply repeatedly as myeloma cell.
- So, isolation of hybrid cell because is only cell which survive in HAT medium.

6. SCREENING OF HYBRIDOMA CELLS

- ELISA screening method which done by incubating hybridoma culture in which secondary enzyme gets conjugate and formation of colored product shows positive hybridoma.
- Used for multiplying the hybridoma cells
  - In-vivo
  - In-vitro


In vivo
In vitro

- In-vivo procedure involves introduction of hybridoma cells into the peritoneal cavity of the animal , then from ascetic fluid antibodies are isolated.
- In-vitro method involves culturing of hybridoma cells in suitable culture media and then antibodies are isolated and purified.
- Once a hybridoma colony is established, it will continually grow in culture medium like RPMI-1640 and produce antibodies.
- Storage: liquid nitrogen.


immunization
myeloma cell culture
antibodies
HGPRT+ Ig+
B cells
HGPRT− Ig−
myeloma cell
fuse cells in PEG
plate cells in HAT selective media
hybrid 1
hybrid 2
hybrid n
myeloma
B lymphocyte
dies
dies
cloning of individual hybridoma cells and testing the clones for production of the desired antibody
selection of the desired antibody producing hybridoma for cloning
tumour induction
mass culture growth
freeze hybridoma for future use
monoclonal antibody
monoclonal antibody

APPLICATION OF HYBRIDOMA TECHNOLOGY

- **Serological:**
  - Identification of ABO blood group
- **Diagnosis:**
  - Detection of pregnancy by assaying of hormones with monoclonal.
  - Separation of one substance from a mixture of very similar molecules.
- **Immunopurification:**
  - Purification of individual interferon using monoclonal.
  - Inactivation of T-lymphocytes responsible for rejection of organ transplants.
- **Therapy:**
  - Removal of tumor cell from bone marrow.
  - Treatment of acute renal failure.
  - Treatment malignant leukemic cells, B cell lymphomas, and a variety of allograft rejections after transplantation.