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Department of Biotechnology
Faculty of Engineering
& Technology

LT.12 Cell Synchronization & Transformation

Content Outline

1. Cell synchronization
2. Methods of cell synchronization
3. Cell synchronization using thymine double block method
4. Confirmation of cell synchronization
5. Transformation (Cellular)
6. Immortalization
7. Mechanism of immortalization

Cell synchronization

•Cell synchronization is a process by which cells at different stages of the cell cycle in a culture are brought to the same phase.

•Through synchronization, cells at distinct cell cycle stage could be obtained.

•Many cell-synchronization protocols involve placing cells under growth inhibitory conditions for periods of time related to the duration of specific cell-cycle phases.

•Cell synchronisation is used to study the progression of cells through the cell cycle

➢Cell can be synchronized in any of the following main stages of cell cycle

G0/G1 phase – cell rest and recovery in preparation for subsequent rounds of cell division

S Phase – DNA replication (interphase)

G2/M phase – chromosome segregation and mitosis

Targeting the cell cycle stage:

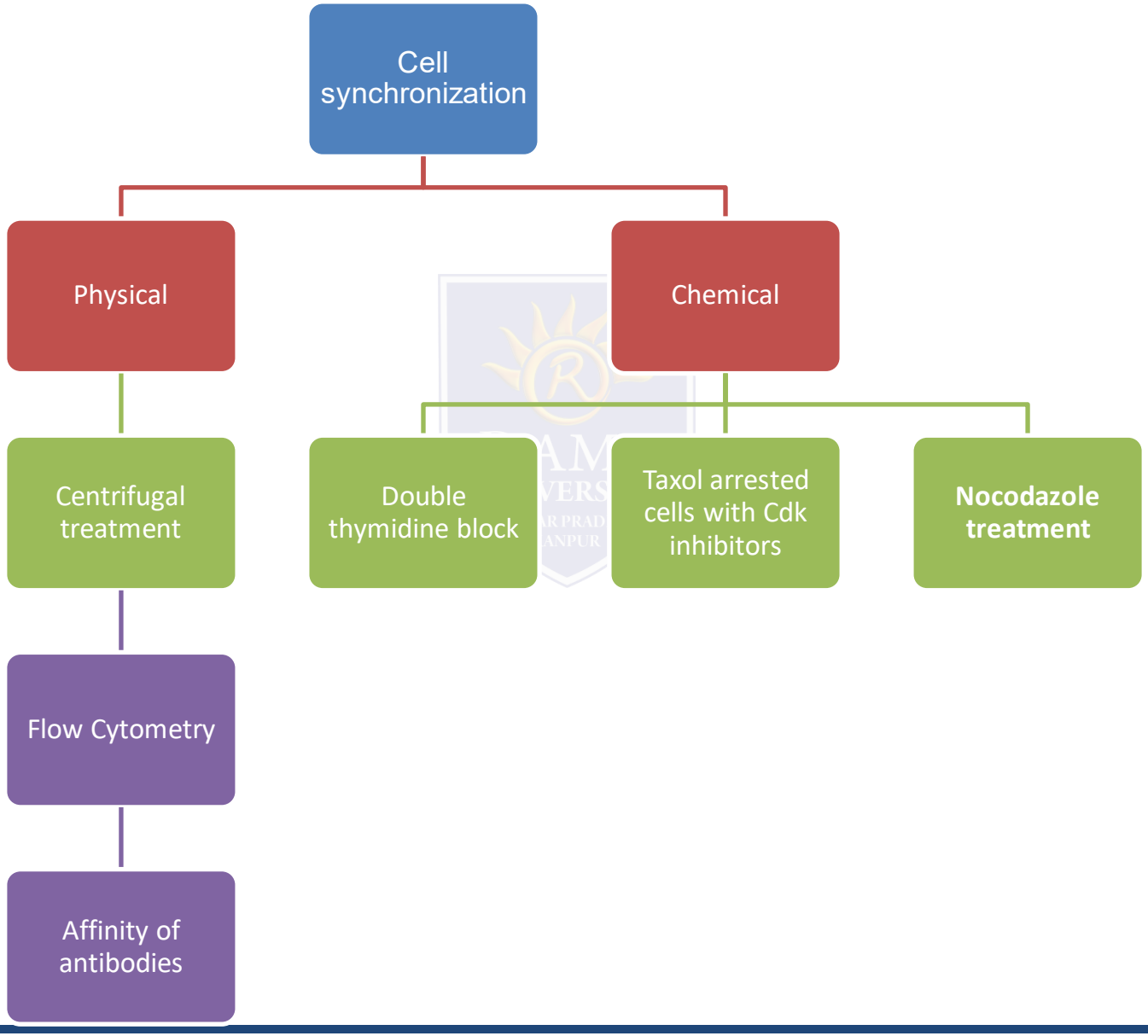
• Release from GO arrest,

•release from M- and S-phase blocking agents,

• mitotic detachment

•centrifugal elutriation

Methods of cell synchronization



Physical methods

Physical Fractionation

•Physical fractionation or cell separation techniques can be based on cell density, cell size, affinity of antibodies on cell surface epitopes and light scatter or fluorescent emission by labeled cells.

•Centrifugal separation or fluoresecene-activated cell sorting are primarily used for physical fractionation. Centrifugal separation enables the separation of cells based on size and sedimentation velocity. Fluorescence-activated cell sorting (FACS) sorts cells on based on differences which can be detected by light scatter (e.g. cell size) or fluorescence emission (by penetrated DNA, RNA, proteins, antigens). This can be carried out using a flow cytometer or fluorescence-activated cell sorter.

Chemical Blockade

•As the names suggests this method uses chemicals such as Thymidine to block metaboli reactions. This can be achieved through inhibition of DNA synthesis largely during the S-Phase o the cell cycle. Inhibitors such as thymidine, aminopterin, hydroxyurea and cytosine arabinoside can have variable effects. Serum starving your cells for 24 hrs will result in an accumulation of cells at G1 phase. The effects of serum starvation can be reversed by the addition of serum to media once cell synchrony has occured.

•Different chemicals can induce cell synchronisation at different stages in the cell cycle

Cell Cycle Stage Targeted	Treatment used
G1 arrest	Double Thymidine block,Serum starvation. Inhibition of cyclin dependent kinase (CDKs)
G2 arrest	Inhibition of microtubules, Inhibition of cyclin dependent kinase (CDKs)
M-Phase	Taxol, Nocodazole

Cell synchronization using double thymidine block

•To synchronise cells at the G1/S border a freshly prepared thymidine solution (16 mM) was made up in complete medium and filter-sterilised using a 0.22 µm filter disc.

•Cells (1 X 10^7) were cultured in 60 ml complete medium containing thymidine (2 mM) for 16 h in a 175 cm^3cell culture flask.

•The cells were then resuspended in complete medium (52.5 ml) for 8 h to allow cells to

• reenter the cell cycle.

•Cells were harvested by centrifugation at 400 x g for 5 min and washed X 2 in complete medium (10 ml). Cells (1 X 10^7) were cultured in 60 ml complete medium containing thymidine (2mM) for 16 h to synchronise cells at the G1/S border.

•Cell synchronisation was confirmed by flow cytometry.

Protocol adopted as it is from
https://www.elisagenie.com/cell-synchronisation-methods/
Also visit this site for other detailed protocol

Confirmation of cell synchronization

•Cell synchronisation can be confirmed by microscopy or flow cytometry. Microscopy allows you to see what is actually going on inside you cells.

•Flow cytometry enables you to compare your treated synchronised cells against a asynchronous control. Briefly the protocol is as follows;

1. Fix and permeabilize your cells in 70 % ethanol
2. Stain with 40 µg/ml propidium iodide, and include 25 µg/ml of Rnase (to degrade RNA and ensure that you stain DNA only).
3. Run your samples on the flow cytometer.

Transformation (Cellular transformation)

•Transformation of cultured cells implies a spontaneous or induced permanent phenotypic change resulting from a heritable change in DNA and gene expression.

•Although transformation can arise from infection with a transforming virus, such as polyoma, or from transfection with genes such as mutant *ras*, it can also arise spontaneously, after exposure to ionizing radiation, or after treatment with chemical carcinogens

•Transformation is associated with genetic instability

Phenotypic changes associated with transformation are:

i. Immortalization

ii. Aberrant growth control

iii. Malignancy

Characteristic of Transformed cells

Genetic instability:

- Cells have high rate of undergoing mutations and changes their genetic make up even with slight induction
- Continuous cell lines, particularly from tumors of all species, are very unstable.
- Deletion or alteration in DNA surveillance genes, such as p53, are usually implicated.

•Continuous cell lines are usually *heteroploid*, meaning they show a wide range in chromosome number among individual cells in the population, implying substantial genetic diversity.

There are two main causes of genetic heterogeneity:

(1) the spontaneous mutation rate appears to be higher in vitro, associated, perhaps, with the high rate of cell proliferation and defective DNA surveillance genes, particularly p53,

(2) mutant cells are not eliminated unless their growth capacity is impaired.

Chromosomal aberration

•Aberration refers to a characteristic that deviates from the normal type

•Chromosomal abnormalities occur when there is either an excess or a loss of a total chromosome or when a portion of a chromosome is absent (macrodeletions and microdeletions).

•Most tumor culture show signs of *aneuploidy*, meaning deviations from the normal complement of chromosomes. Some specific aberrations are associated with particular types of malignancy

➢As result of chromosomal aberrations, the transformed cells show anchorage independence, loss of contact inhibition, and low serum requirement.

Anchorage independence

There occur several changes on the cell surfaces of transformed cells. These include alterations in the cell surface glycoproteins and integrin's, and loss of fibronectin. Some of the transformed cells may totally lack cell adhesion molecules (CAMs). The modifications on the surface of transformed cells leads to a decrease in cell—cell, and cell- substrate adhesion.

Contact inhibition:

The transformed cells are characterized by loss of contact inhibition. This can be observed by the morphological changes in the disoriented and disorganized monolayer cells. This result in a reduced density limitation of growth, consequently leading to higher saturation density compared to normal cells.

Low serum requirement:

In general, transformed cells or tumor cells have lower serum dependence than the normal cells. This is mostly due to the secretion of autocrine growth factors by the transformed cells

Immortalization of cell

•Most normal cells have a finite life span of 20 to 100 generations, but some cells, notably those from rodents and from most tumors, can produce continuous cell lines with an infinite life span.

•These dominantly acting genes synthesize products which inhibit the cell cycle progression.

•It is strongly believed that immortalization occurs due to inactivation of some of the cell cycle regulatory genes e.g. Rb, p^{53} genes.

•Out of 10 genes involved in senescence, some of gene s owing to mutation negatively regulate the expression of telomerase, required for the terminal synthesis of telomeric DNA, which otherwise becomes progressively shorter during a finite life span, until the chromosomal DNA can no longer replicate.

Mechanism of Immortalization

➢It has been assumed that immortalization is a multistep process involving the inactivation of a number of cell cycle regulatory genes, such as Rb and p53. The SV40 LT gene is often used to induce immortalization. The product of this gene, T antigen, is known to bind Rb and p53. By doing so, it not only allows an extended proliferative life span but also restricts the DNA surveillance activity of genes like p53, thereby allowing an increase in genomic instability and an increased chance of generating further mutations favorable to immortalization (e.g., the upregulation of telomerase or the downregulation of one of the telomerase inhibitors). Transfection of the telomerase gene with a regulatable promoter is sufficient to immortalize cells

Methods of Immortalization: Making cells immortal

Several methods exist for immortalizing mammalian cells in culture conditions. With years of experience in cell immortalization. Recombinant lentiviral, retroviral (MMLV) and adenoviral viruses expressing EBV, HPV-16 E6/7 and SV40 T antigens, hTERT, p53 and RB siRNAs, and ras & myc mutants. All these tools will make your cell immortalization project simpler and easier than ever before.

Follow link for process of immortalization: https://www.biocat.com/cell-biology/cell-immortalization

Test your Understanding

1.Cellular Transformation refers to

a. Transforming cells by inserting exogenous DNA
b. Transforming cells through alteration of endogenous DNA
c. Both (a) and (b)
d. None of the above

2.Centrifugal elutriation is used for

a. Cell synchronization
b. Sorting of cells
c. Both (a) and (b)
d. None of the above

3.What happens to cells if p53 and Rb genes are inactivated

a. Cells dies instantaneously
b. Cell undergo apoptosis
c. Cell becomes immortal
d. None of the above

4.Anchorage independency is the characteristics of……..cells.

5. Starvation of cells of nutrient is one method of synchronizing cellular growth in culture. (True / False)

References

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