Cell Viability, Cytotoxicity, Assays for Viable Cells & Hayflick's Phenomenon

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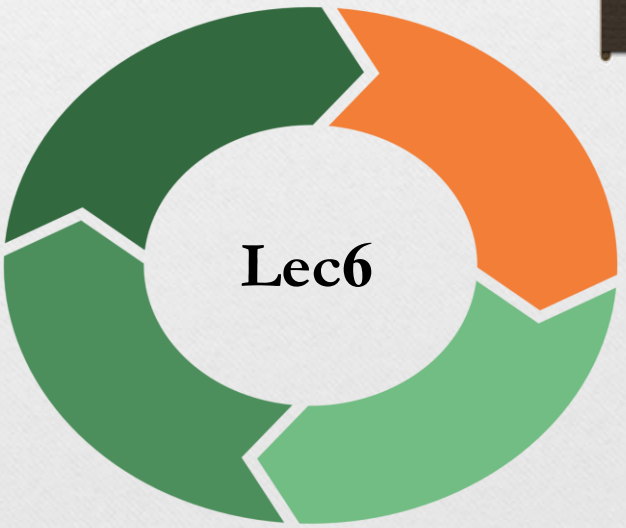


Cell viability

- **Cell viability refers to the ability of cells to survive, grow, and function normally within their environment. It's a fundamental measure in biological research, reflecting the overall health and metabolic activity of cell populations.**

Cytotoxicity describes the capacity of substances or environmental conditions to damage or kill cells through various mechanisms

Why Measure Cytotoxicity?
لماذا نقيس السمية الخلوية؟

1. ◦**Therapeutic Discovery الاكتشاف العلاجي** : Identifying agents that **selectively eliminate harmful** cells, particularly in cancer therapy development, whilst sparing healthy tissue

2. **Safety Assessment تقييم السلامة** :Ensuring pharmaceutical compounds and chemicals don't produce harmful effects on healthy cells during drug development and toxicology studies

Why Measure Cytotoxicity?

3◦**Mechanism Understanding فهم الآلية:**

Distinguishing between different modes of cell death—programmed apoptosis versus uncontrolled necrosis—to understand cellular responses

Introduction

cell viability and cytotoxicity are fundamental concepts in cell biology and toxicology. They are essential for evaluating cell health, proliferation, response to drugs, and environmental stressors. Understanding the assays used to measure cell viability and death is crucial in research, biotechnology, and pharmaceutical applications

Cell Viability
بقاء الخلية

Cell viability refers to the ability of cells to:

Survive

Grow

Maintain metabolic activity

perform normal physiological functions

Factors Affecting Cell Viability

Temperature

pH changes

Nutrient availability

Toxic substances

Oxidative stress

Radiation

Cytotoxicity
السمية الخلوية

Cytotoxicity is the degree to which a chemical, drug, or environmental factor can damage or kill cells

Mechanisms of Cytotoxicity

Cell membrane damage

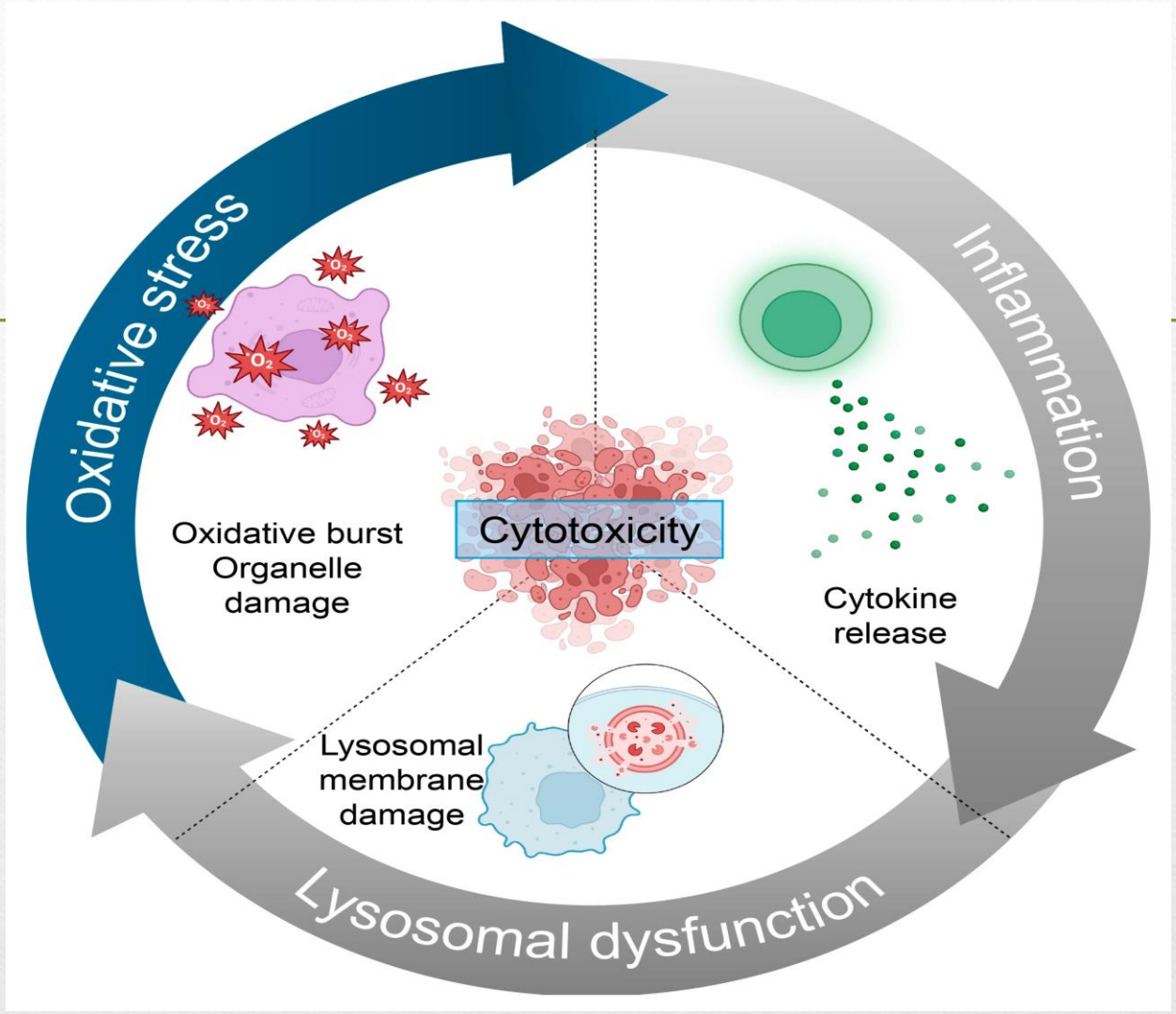
Mitochondrial dysfunction

Oxidative stress (ROS)

DNA damage

Protein misfolding

Activation of apoptosis or necrosis pathways


Oxidative stress
Inflammation
Lysosomal dysfunction
Oxidative burst
Organelle
damage
Cytotoxicity
Cytokine
release
Lysosomal
membrane
damage

Importance

Drug screening

Toxicology testing

Cancer research

Assessment of environmental pollutants

Biomaterial safety testing

Assays to Measure Cell Viability and Cytotoxicity
اختبارات لقياس قابلية الخلايا للبقاء والسمية الخلوية

there are several assays used depending on what aspect of cell health is being evaluated (membrane integrity, metabolism, proliferation, etc.)

Metabolic Activity Assays
اختبارات النشاط الأيضي

- **These detect the activity of cellular enzymes that are active only in viable cells:**

(a) MTT Assay

Measures mitochondrial enzyme activity (reduction of MTT to formazan)

Colorimetric (purple product).

High accuracy, commonly used.

(b) XTT or WST-1 Assays

Similar to MTT but water-soluble (no need for solubilization)

Faster and less toxic

(c) Resazurin Assay (Alamar Blue)

Based on reduction of resazurin to resorufin

Fluorescent and non-toxic

Membrane Integrity Assays
اختبارات سلامة الغشاء

- **(a) Trypan Blue Exclusion Test**

Viable cells exclude dye; dead cells take it up.

Simple and widely used.

(b) LDH Release Assay

LDH is released when the cell membrane is damaged

Measures cytotoxicity indirectly

ATP-Based Assays

ATP Luminescence Assay

(a) Measures ATP concentration as an indicator of metabolically active cells

(b) Highly sensitive

Apoptosis and Necrosis Assays
اختبارات موت الخلايا المبرمج والنخر

(a) Annexin V / PI Staining

Annexin V binds to phosphatidylserine (early apoptosis)

PI stains necrotic or late-apoptotic cells

(b) Caspase Activity Assays

Measures apoptosis-specific enzyme activation

Cell Proliferation Assays
اختبارات تكاثر الخلايا

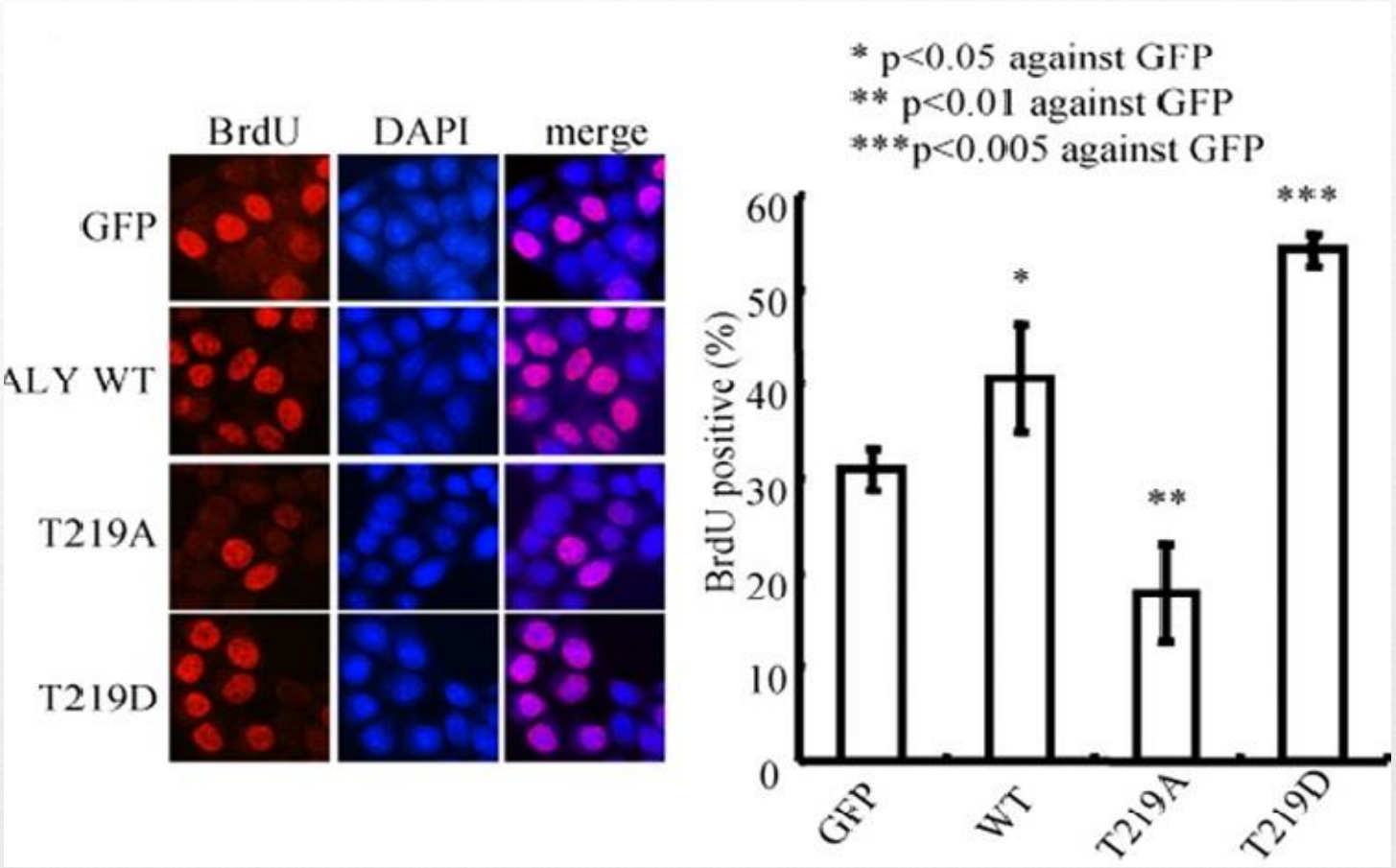
(a) BrdU Incorporation Assay

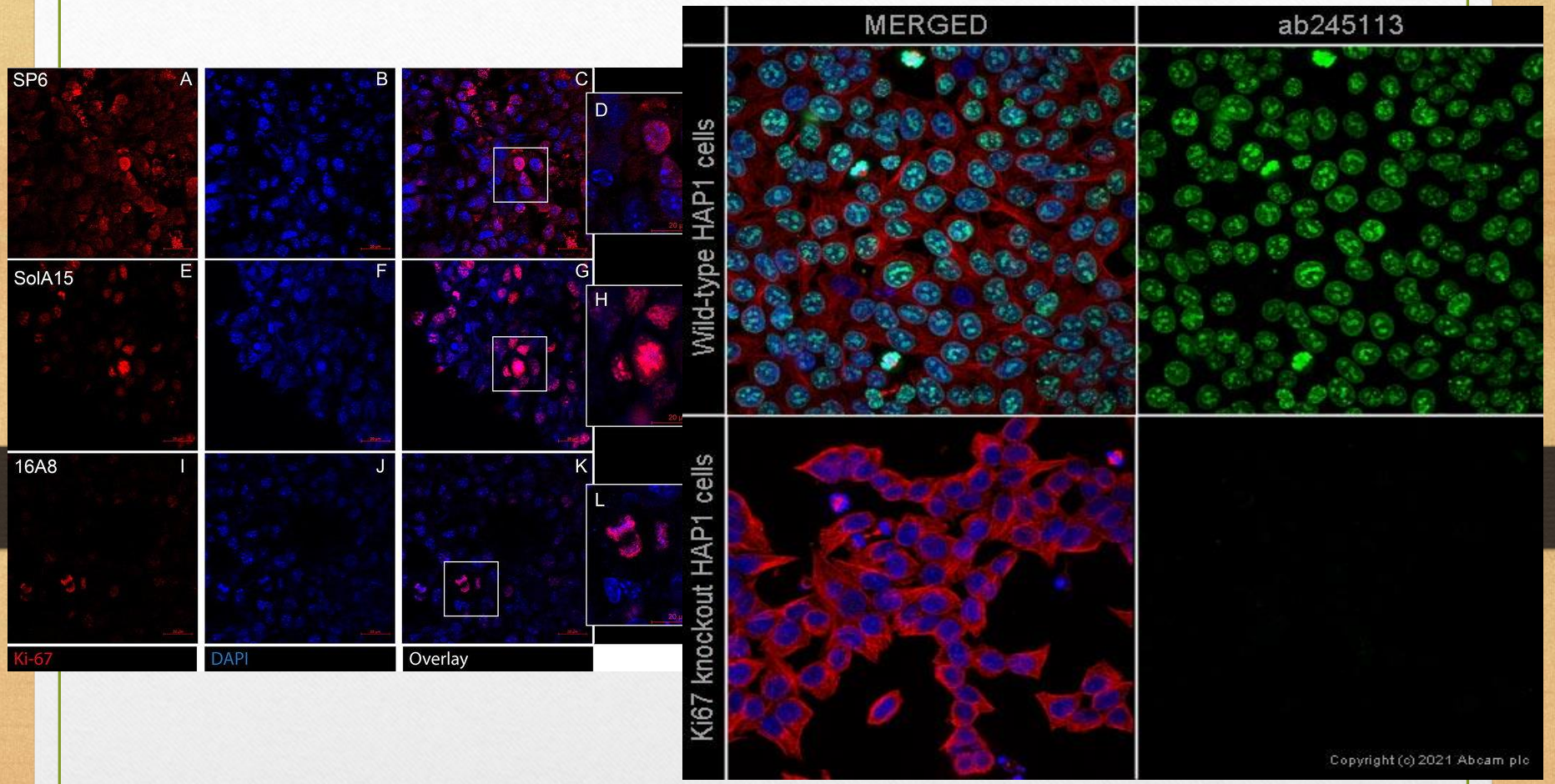
Detects DNA synthesis

Indicates actively dividing cells

(b) Ki-67 Immunostaining

Proliferation marker protein


BrdU
DAPI
merge
GFP
ALY WT
T219A
T219D
* p<0.05 against GFP
** p<0.01 against GFP
***p<0.005 against GFP
BrdU positive (%)
60
50
40
30
20
10
0
*
**
***
GFP
WT
T219A
T219D


SP6
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Overlay
MERGED
ab245113
Wild-type HAP1 cells
Ki67 knockout HAP1 cells
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Hayflick's Phenomenon
ظاهرة هايفليك

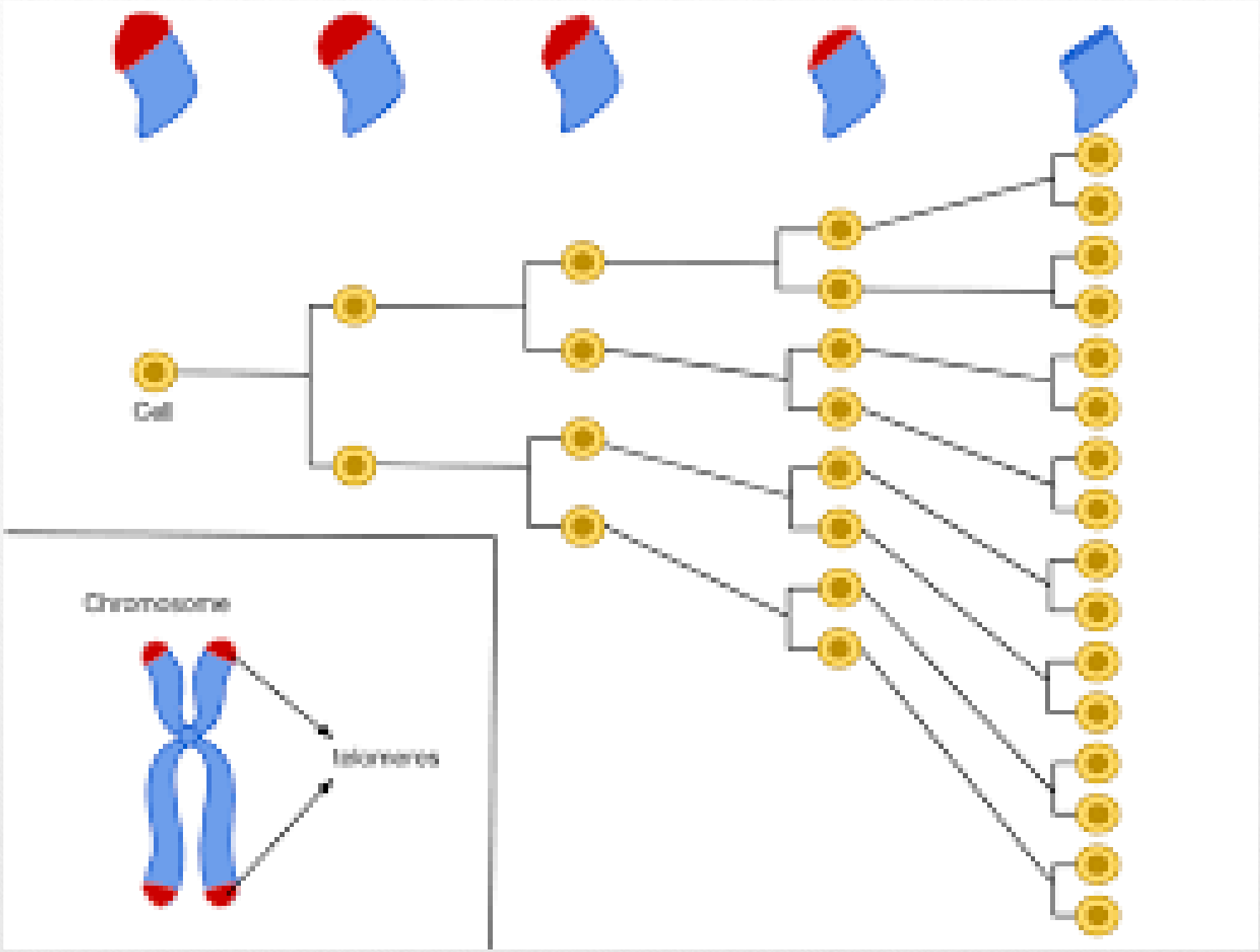
Hayflick's Phenomenon (Hayflick Limit) describes the observation that normal human somatic cells can divide only a finite number of times, typically 40–60 cell divisions in culture

Why do cells have a limited number of divisions?

Telomere Shortening

Each time a cell divides, telomeres (chromosome ends) shorten

When telomeres become too short, the cell enters senescence and stops dividing.


Cell
Chromosome
telomeres

Biological Significance

Explains aging at the cellular level

Prevents unlimited cell division (a cancer-protective mechanism)

Stem cells and cancer cells bypass this limit via telomerase enzyme

Applications

Aging research

Cell culture maintenance

Cancer biology

Regenerative medicine