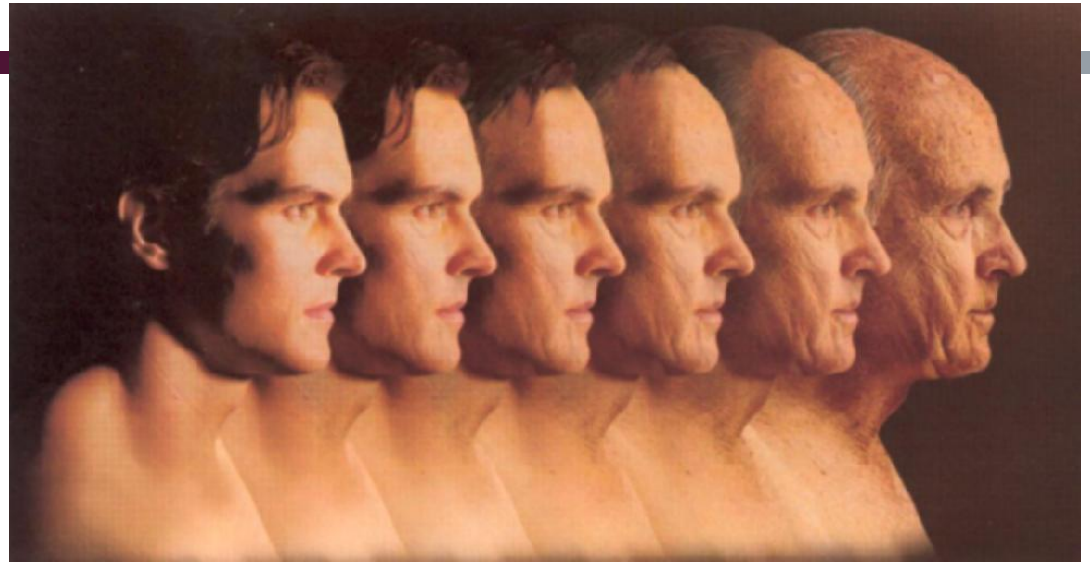
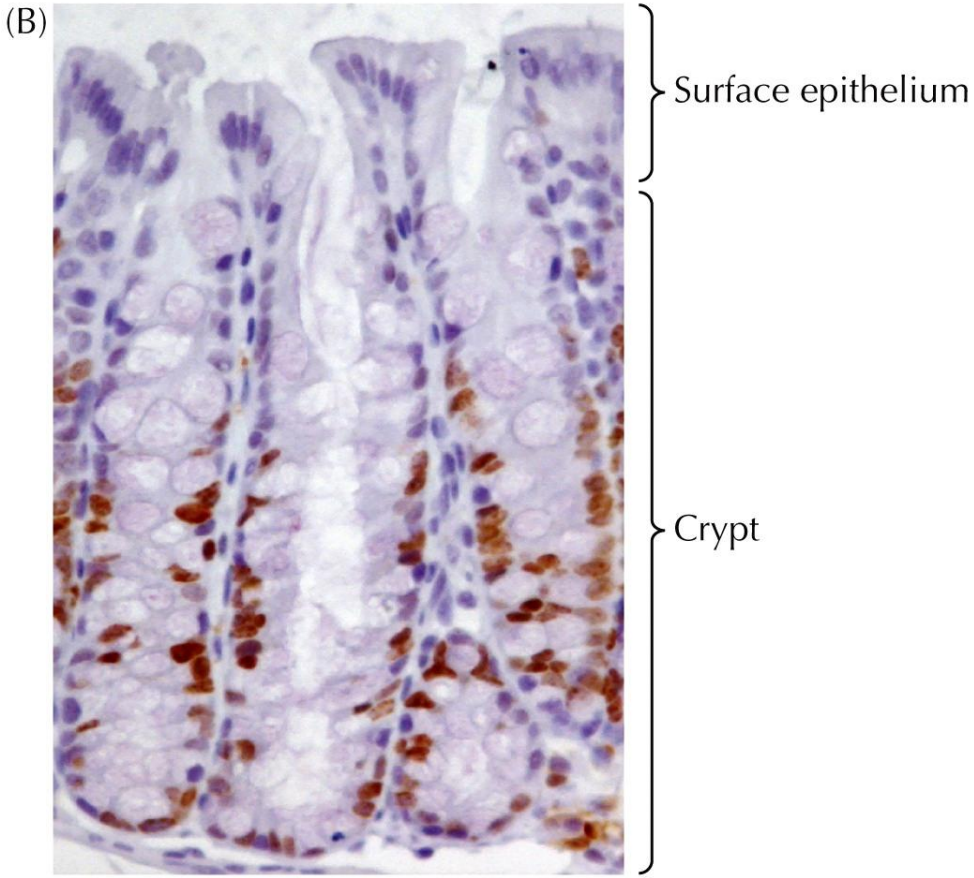


# SENESCENCE AND IMMORTALIZATION

THE SECRET TO STAYING A YOUNG, HIP CELL FOREVER

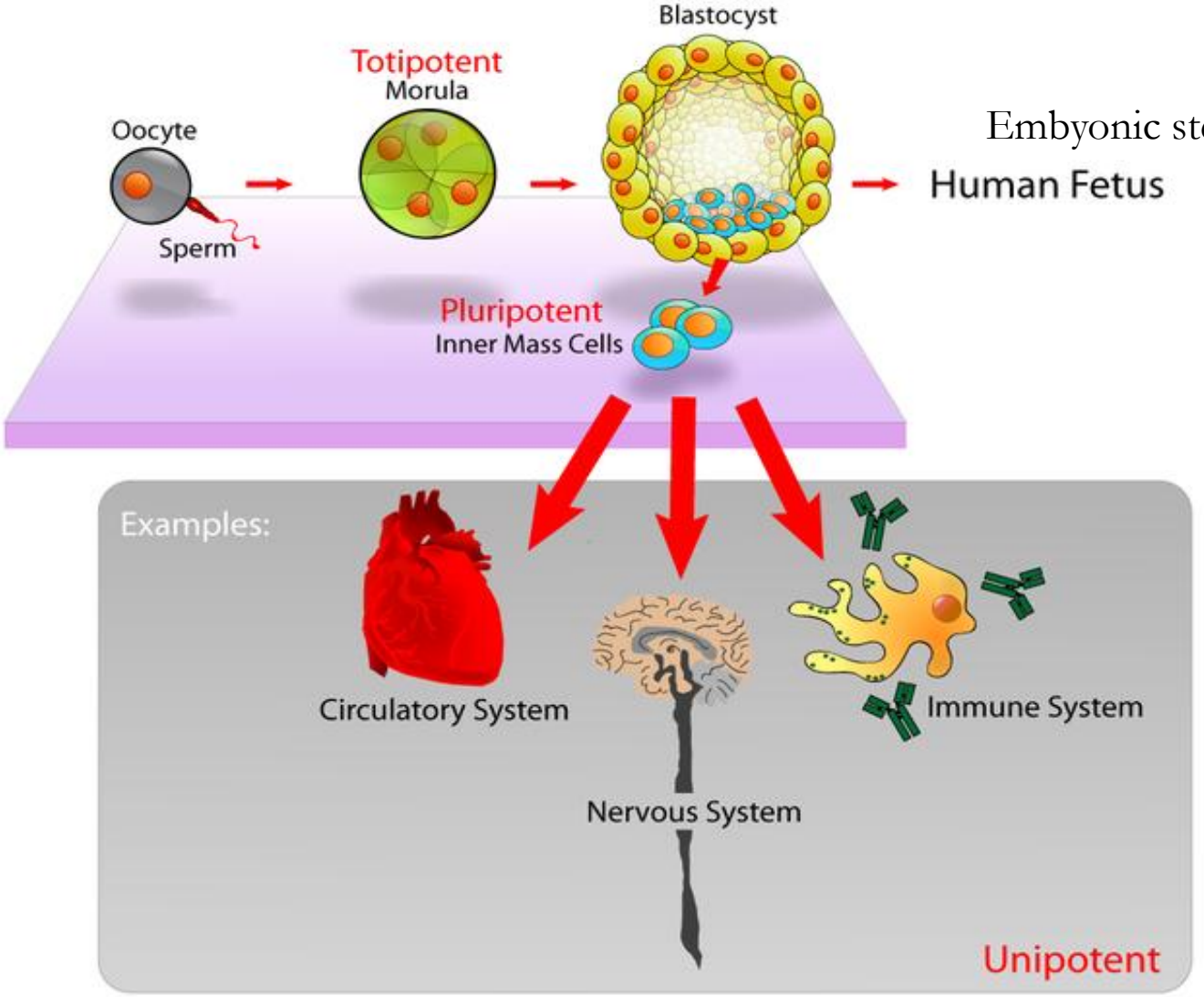
# IMMORTAL CELL SOURCES



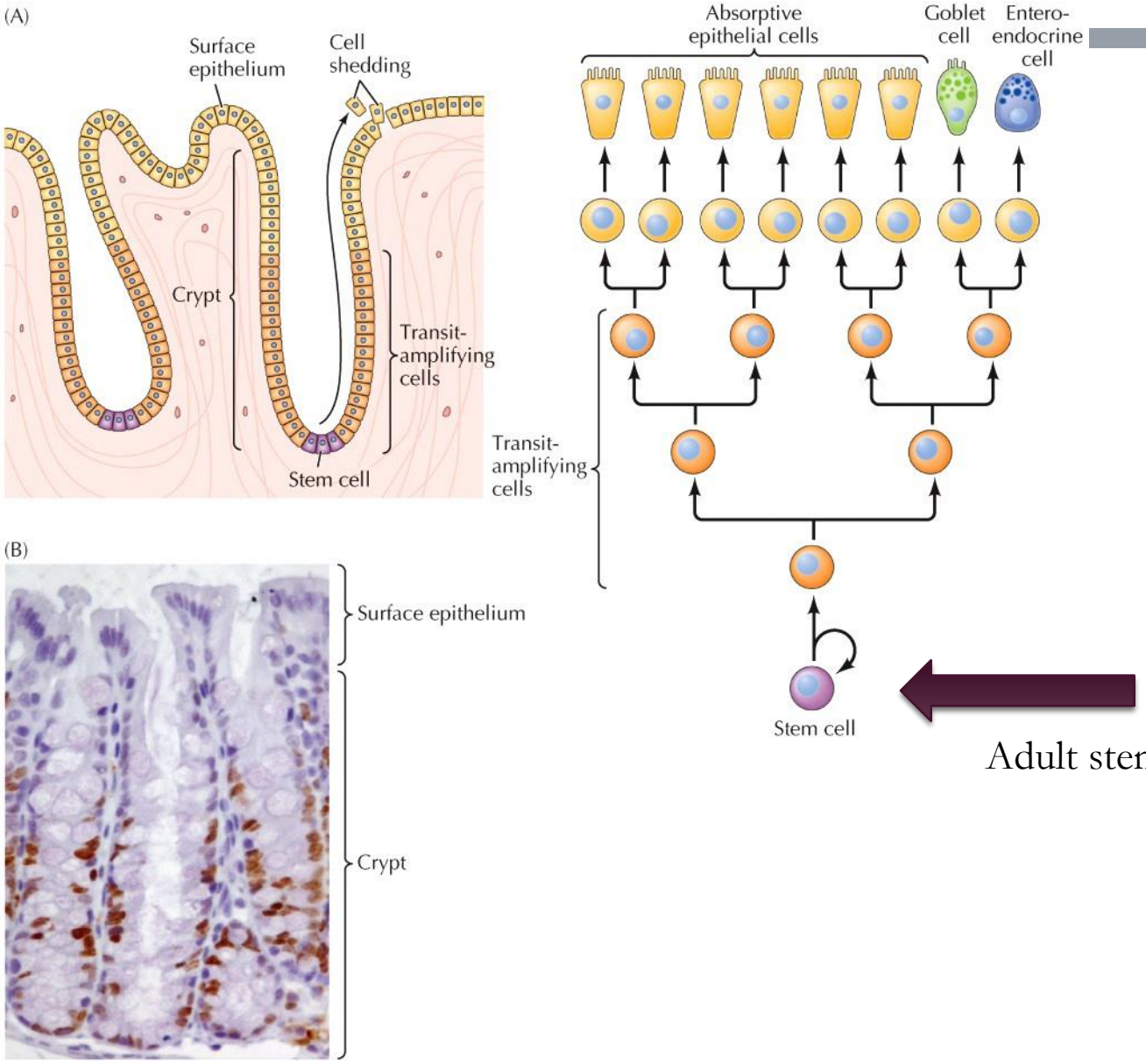

***THE CELL: A MOLECULAR APPROACH 6e*, Figure 17.18 (Part 3)**

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Embyonic stem cells




http://en.wikipedia.org/wiki/File:Stem_cells_diagram.png

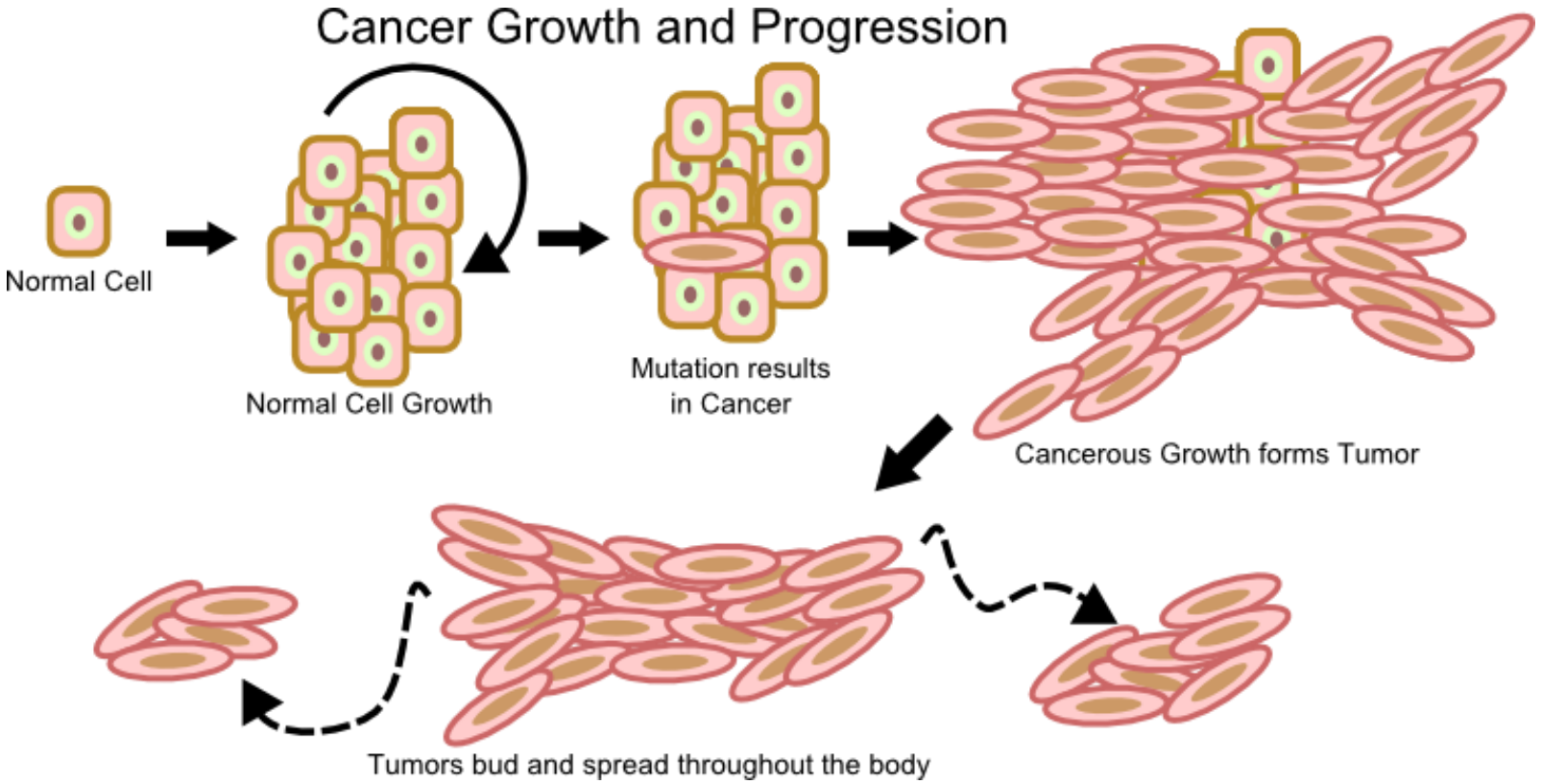


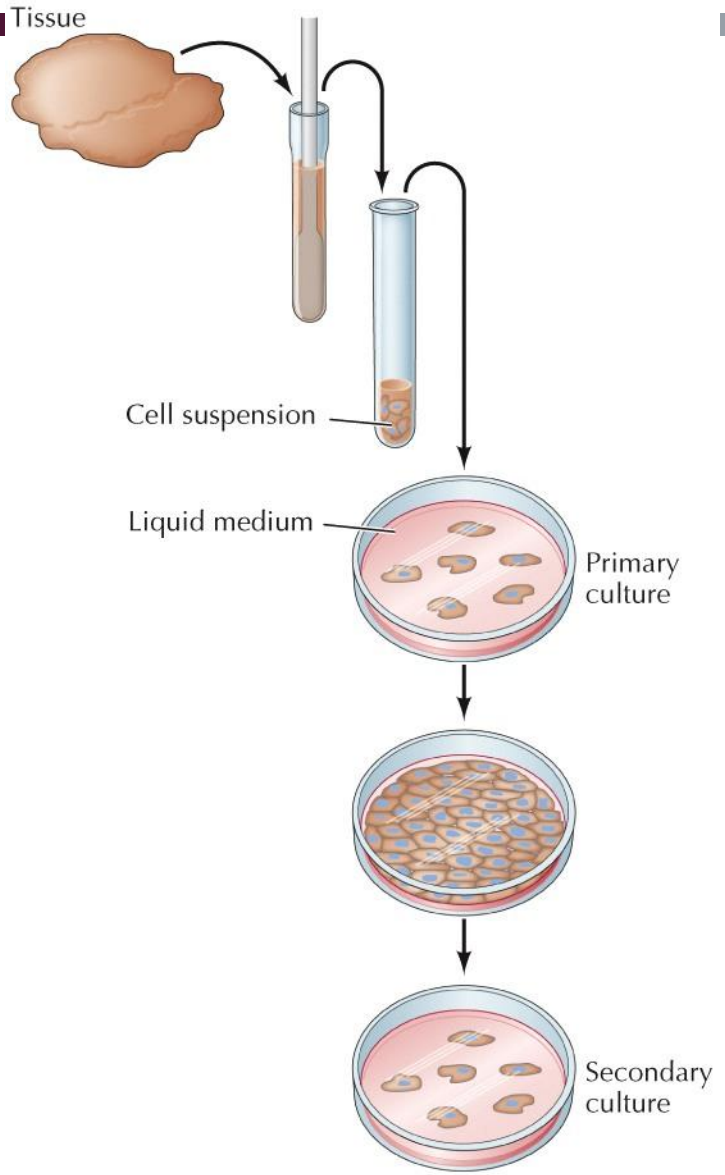

Adult stem cells

***THE CELL: A MOLECULAR APPROACH 6e*, Figure 17.18**

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# Many cell lines come from cancer cells





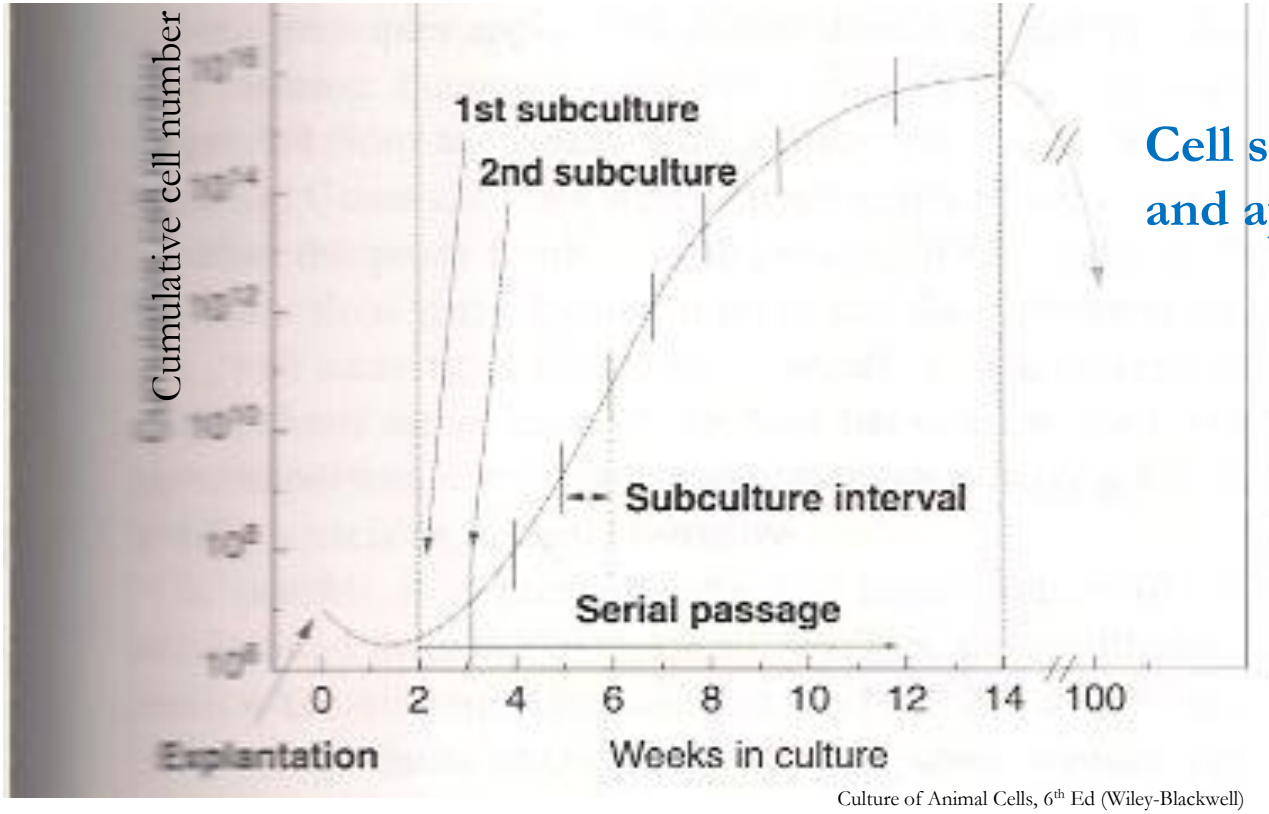

Enzymatic dissociation

Cell culture

***THE CELL: A MOLECULAR APPROACH 6e*, Figure 1.42**

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# Normal eukaryotic cells will not divide indefinitely and are, therefore, not immortal.




Culture of Animal Cells, 6th Ed (Wiley-Blackwell)

**Cell senescence and apoptosis**

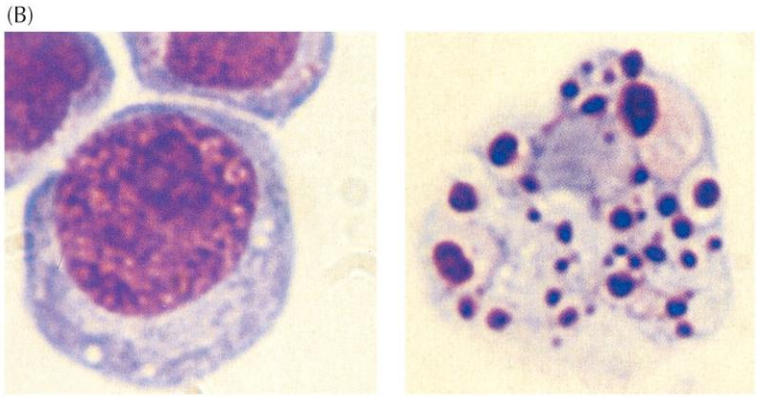
# SENESCENCE and PROGRAMMED CELL DEATH

**Cellular Senescence**

*Def:* The phenomenon by which normal (diploid) eukaryotic cells cease to divide but remain viable. Generally 50 cell divisions in vitro (Hayflick, 1961).

**Programmed Cell Death**

*Def:* A carefully regulated cell death undertaken through apoptosis in response to intracellular signals. Inducers of PCD include virus-infected cells, DNA damage and elimination of unnecessary cells during development.




THE CELL: A MOLECULAR APPROACH 6e, Figure 17.1 (Part 2)
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# SENESCENCE and PROGRAMMED CELL DEATH

## TELOMERES

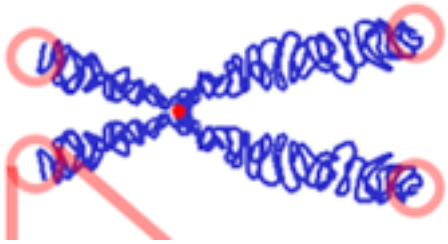
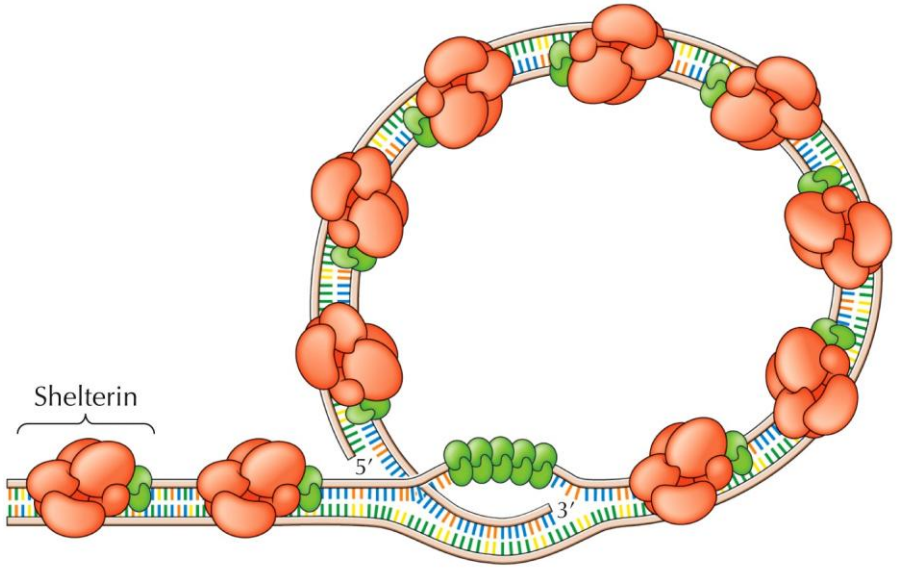


**TABLE 5.6 Telomeric DNAs**

| Organism | Telomeric repeat sequence |
|---|---|
| Yeasts | |
| *Saccharomyces cerevisiae* | $TG_{1-3}$ |
| *Schizosaccharomyces pombe* | $TTACG_{2-5}$ |
| Protozoans | |
| *Tetrahymena* | TTGGGG |
| *Dictyostelium* | $AG_{1-8}$ |
| Plant | |
| *Arabidopsis* | TTTAGGG |
| Mammal | |
| Human | TTAGGG |

*THE CELL: A MOLECULAR APPROACH 6e*, Table 5.6

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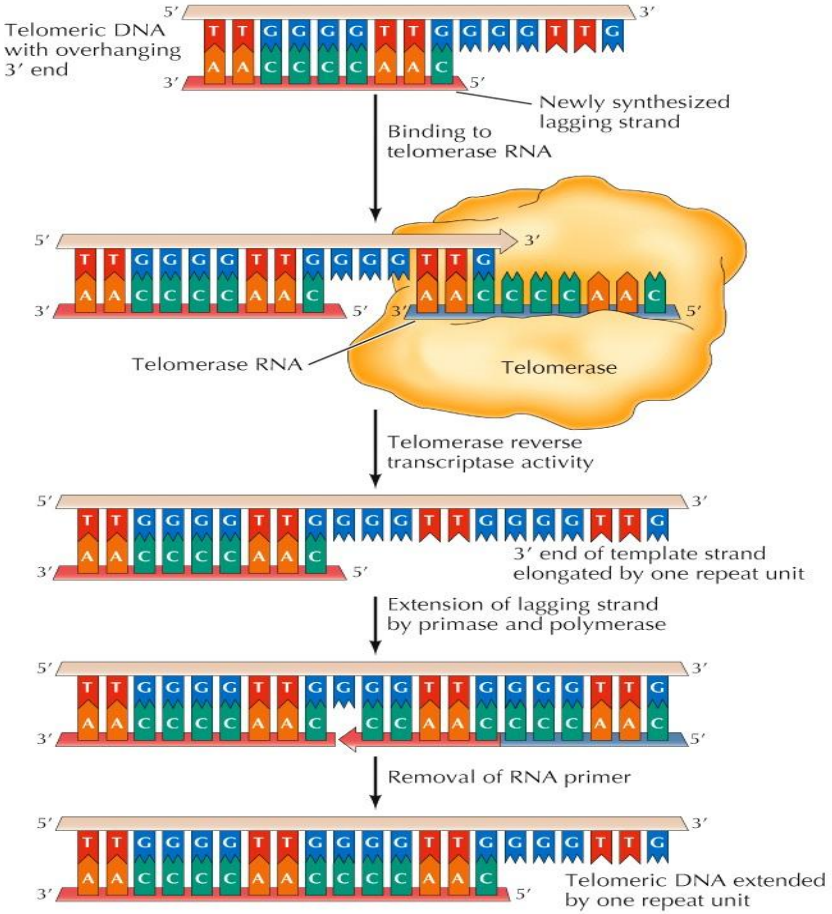



*THE CELL: A MOLECULAR APPROACH 6e*, Figure 5.26

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# SENESCENCE and PROGRAMMED CELL DEATH

## TELOMERES

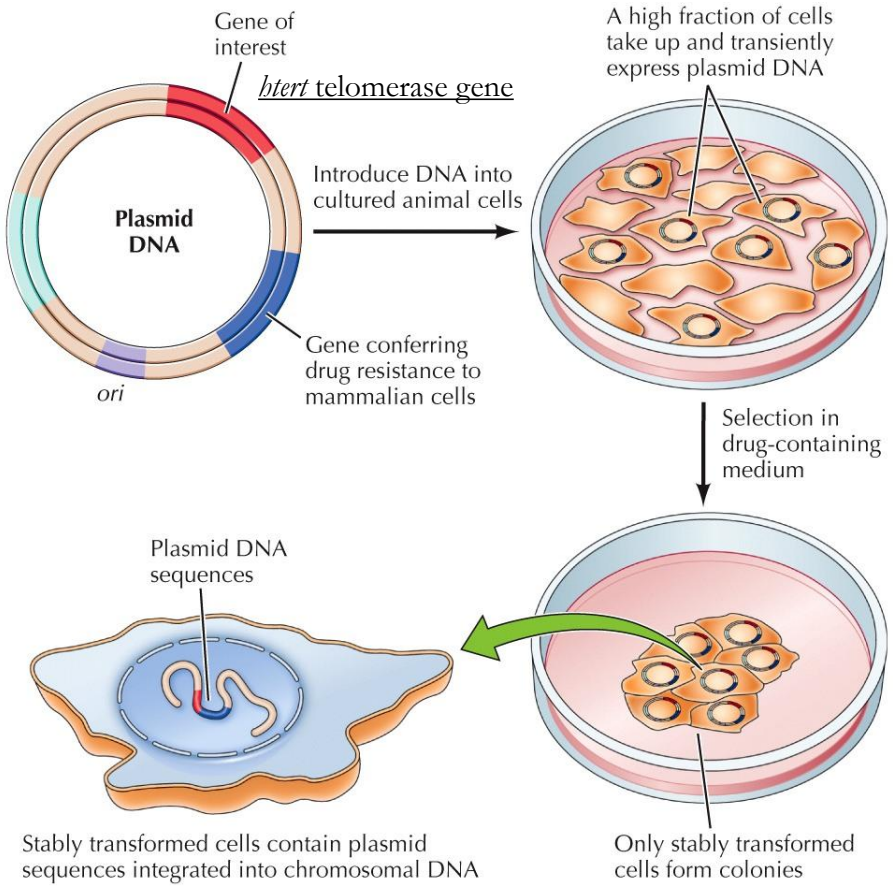



***THE CELL: A MOLECULAR APPROACH 6e*, Figure 6.16**

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Telomerase

# Artificial Immortalization




***THE CELL: A MOLECULAR APPROACH 6e*, Figure 4.32**

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We can make cells immortal by adding exogenous htert.

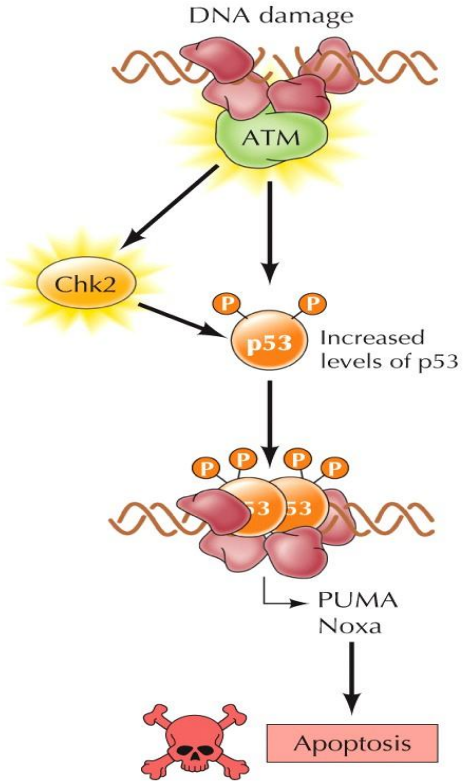
Telomerase is a holoenzyme and htert is the limiting factor.

Bodnar, A. G., et al (1998). Extension of life-span by introduction of telomerase into normal human cells. *Science* **279**:349-352.

# REGULATION OF SENESCENCE AND PCD

## CELL CYCLE REGULATORS

**P53** and **Rb** proteins inhibits cells which have DNA damage



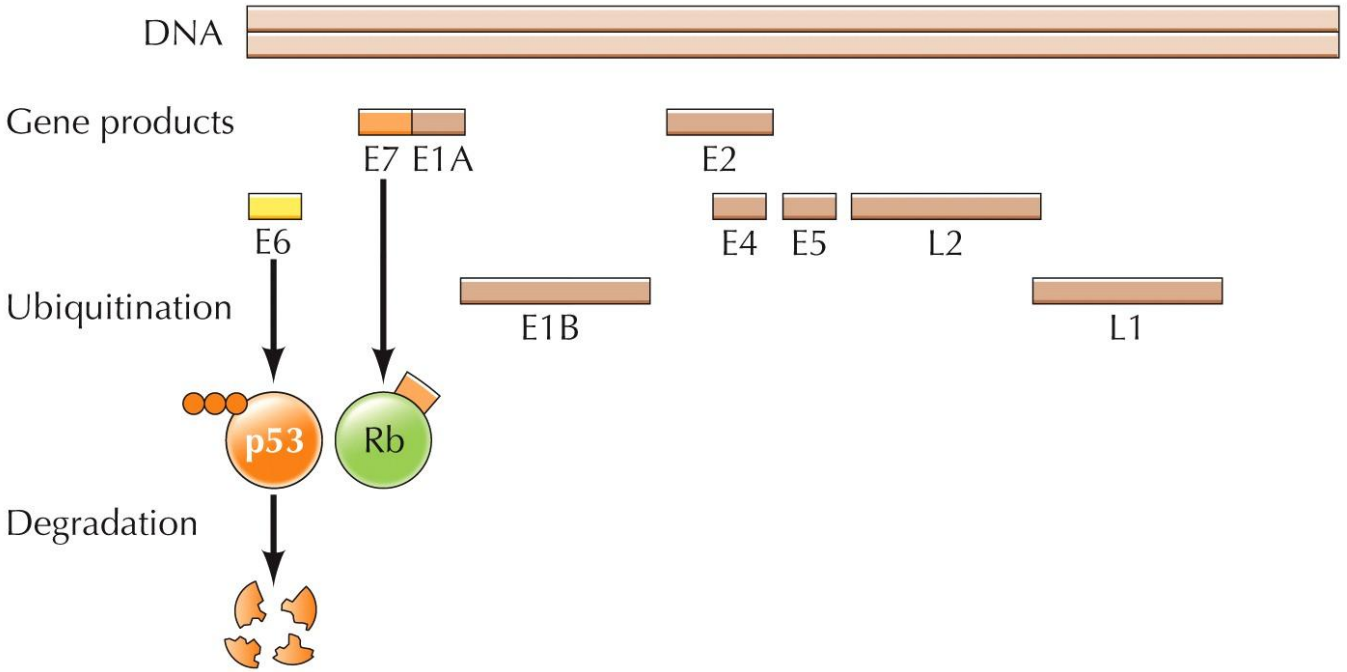

*THE CELL: A MOLECULAR APPROACH 6e*, Figure 17.9

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# REGULATION OF SENESCENCE AND PCD

## CELL CYCLE REGULATORS

Many cancers arise from a mutation that inhibits expression of these cell cycle regulators.

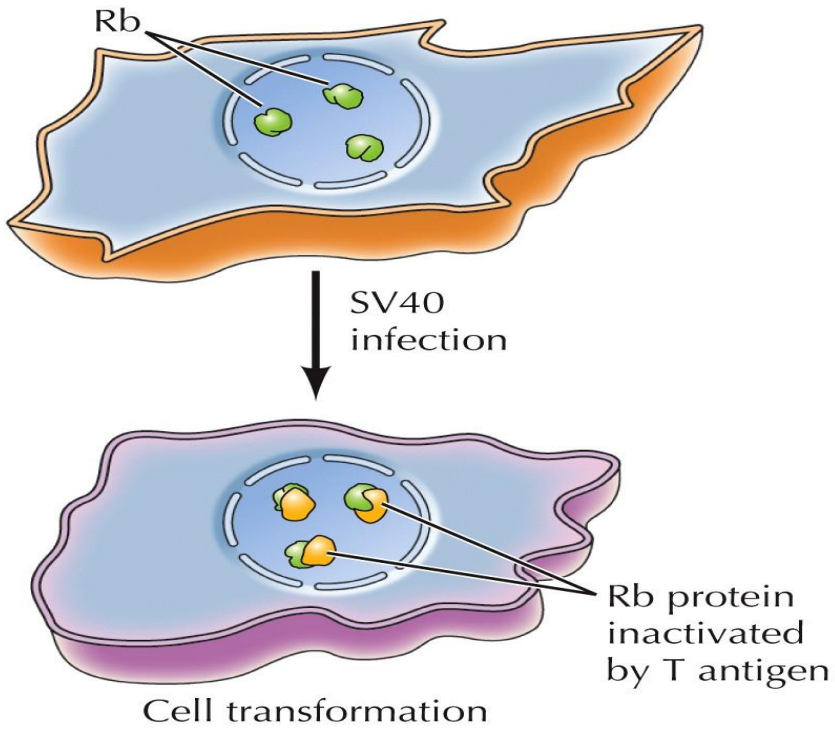



***THE CELL: A MOLECULAR APPROACH 6e*, Figure 18.13**

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# Artificial Immortalization

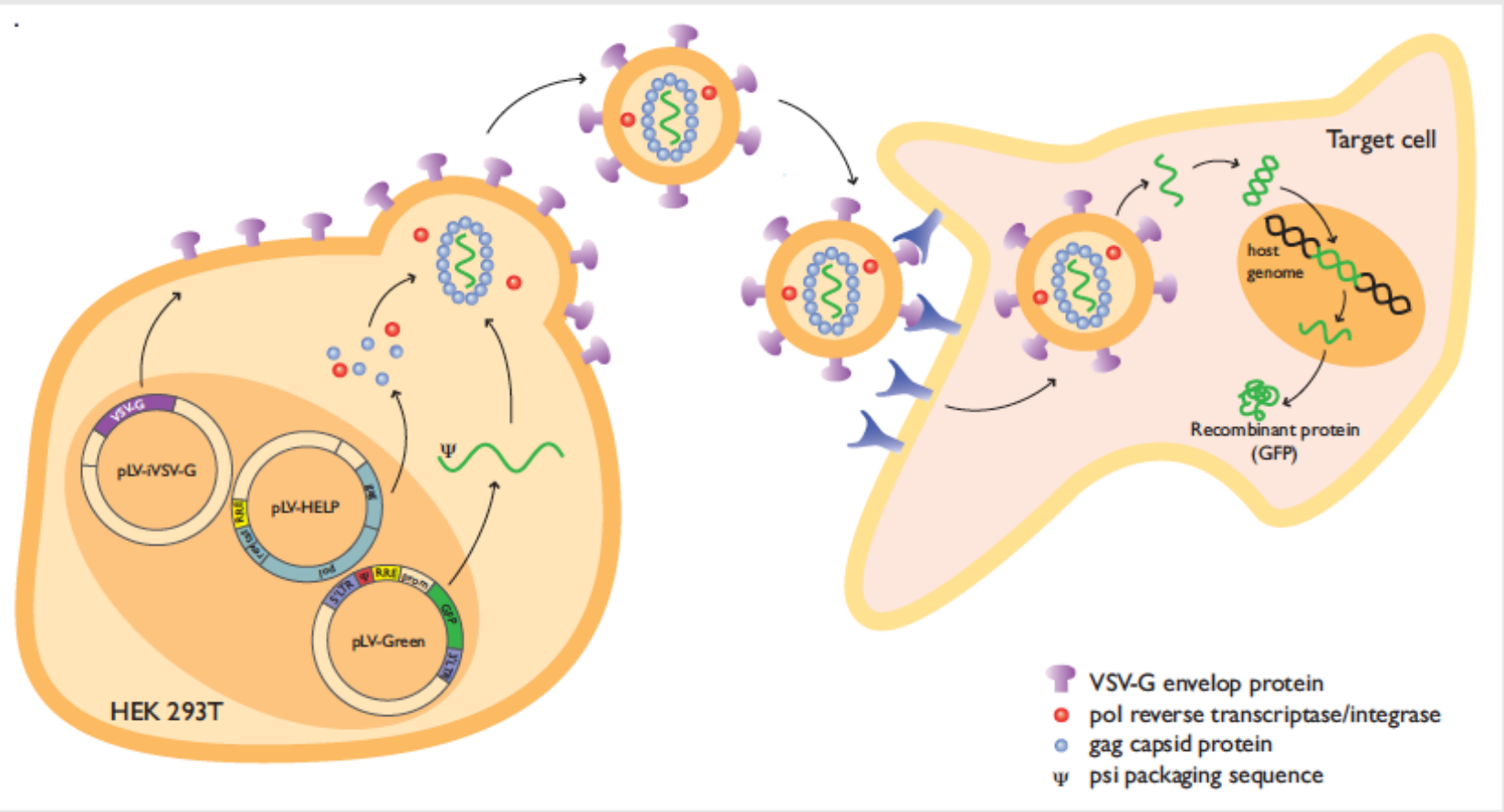
Culture cells can be transfected with the SV40 virus, which expresses a gene responsible for inhibiting p53 and Rb

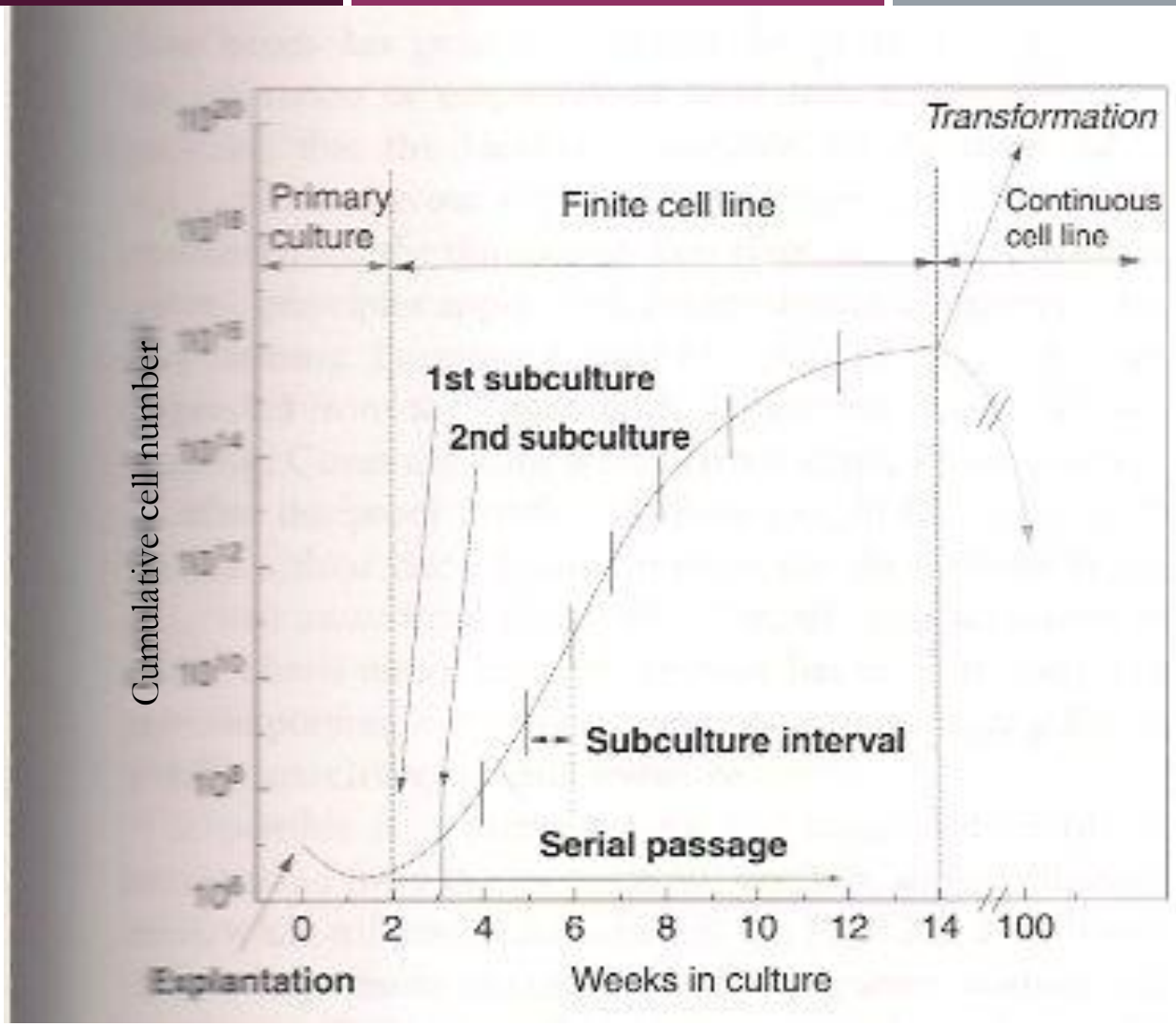



***THE CELL: A MOLECULAR APPROACH 6e*, Figure 18.33**
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# Artificial Immortalization

## Viral Infection

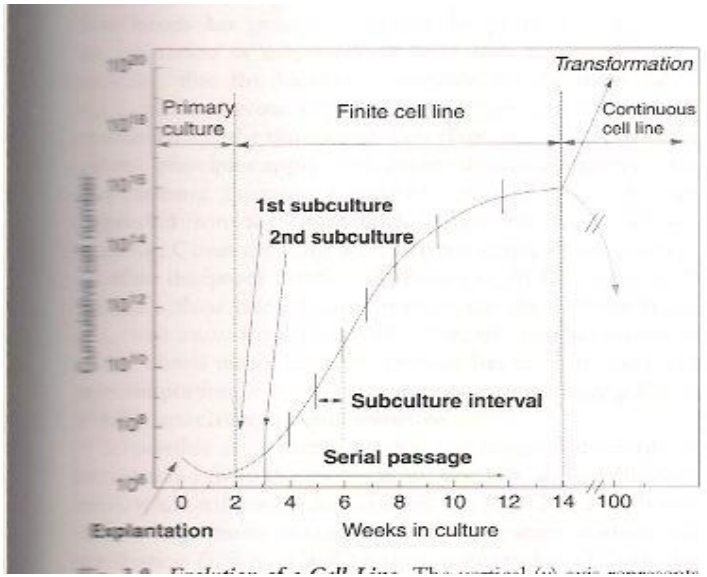




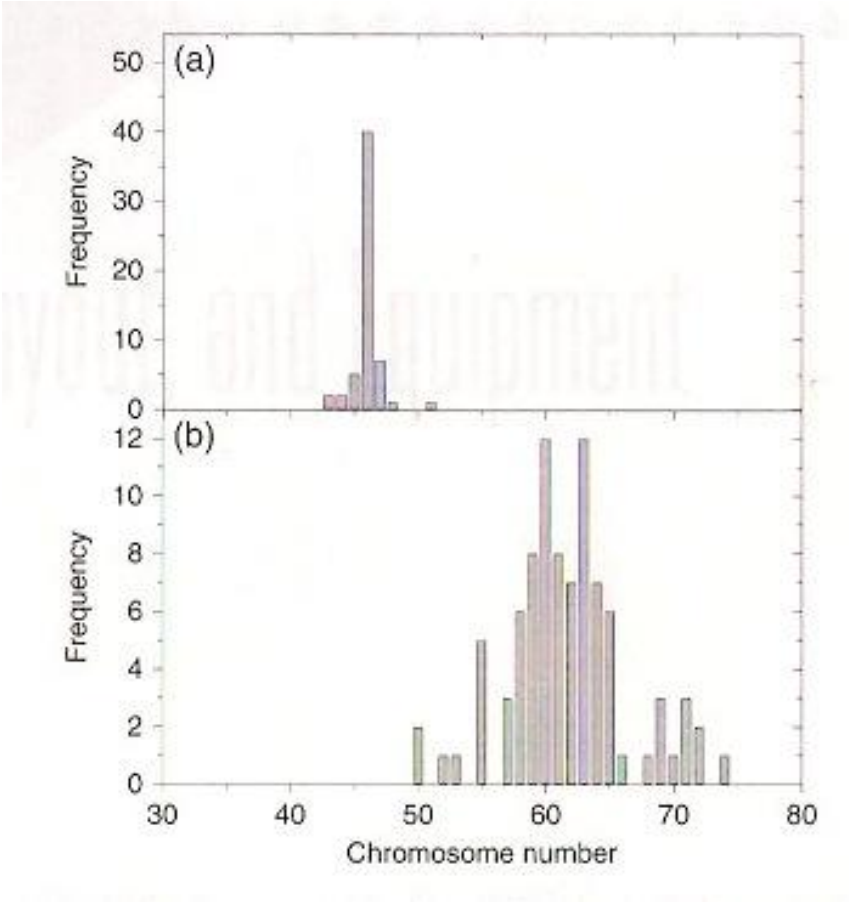

Culture of Animal Cells, 6th Ed (Wiley-Blackwell)

# Take home point: How to get an immortal (continuous) cell line…

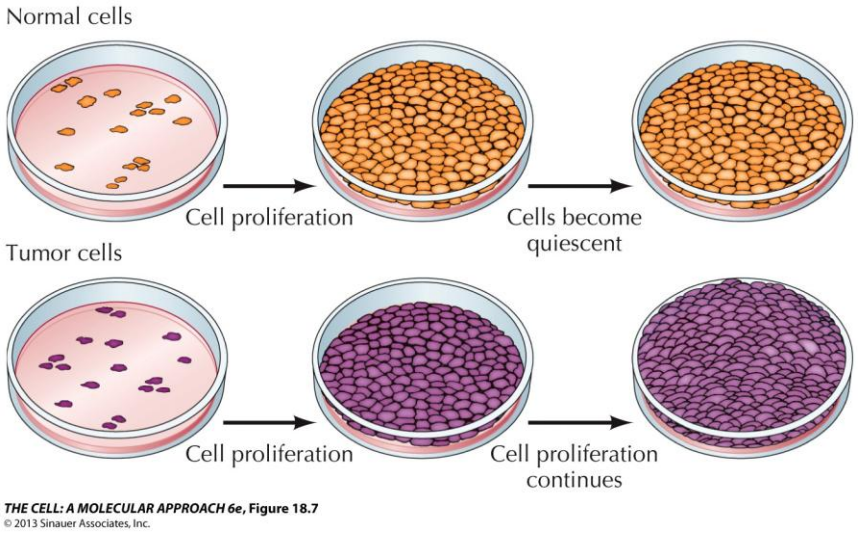
1. **Stem cells** (or induced pluripotent stem cells… more later)
2. **Neoplastic (cancer) cells**
3. **Transformed cells**
    1. Transfected cells with increased telomerase
    2. Transfected cells with decreased cell cycle regulators
    3. Luck! Genetic instability and spontaneous mutation



# Are these cells "normal"?? Probably not!




Culture of Animal Cells, 6th Ed (Wiley-Blackwell)




*THE CELL: A MOLECULAR APPROACH 6e*, Figure 18.7
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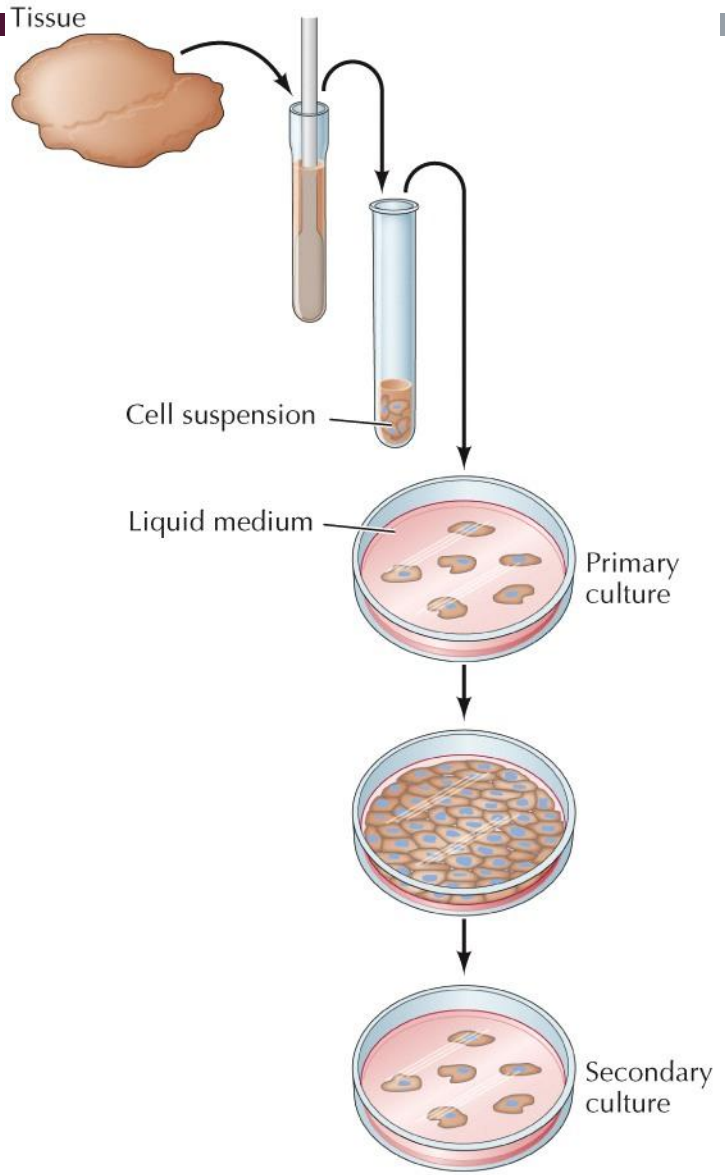
The line between immortality and neoplasia is often very thin!



# SUBCULTURE


I was a hipster...
Before it
was cool.
yourecards
someecards.com


SUBCULTURES
When you don't want to
be like everyone else.




Enzymatic dissociation

Cell culture

***THE CELL: A MOLECULAR APPROACH 6e*, Figure 1.42**

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A **subculture** is a new culture made by transferring some of the cells from a previous culture to a new flask with fresh growth media. Subculturing (also known as cell passage) prolongs the life of cells by allowing them to continue dividing.

# CRITERIA FOR SUBCULTURE

**(1) Density of culture**

- Cells should be subcultured as soon as they reach confluence

**(2) Exhaustion of medium**

- Indicates that the medium requires replacement, but often accompanies an increase in cell density

**(3) Time since last subculture**

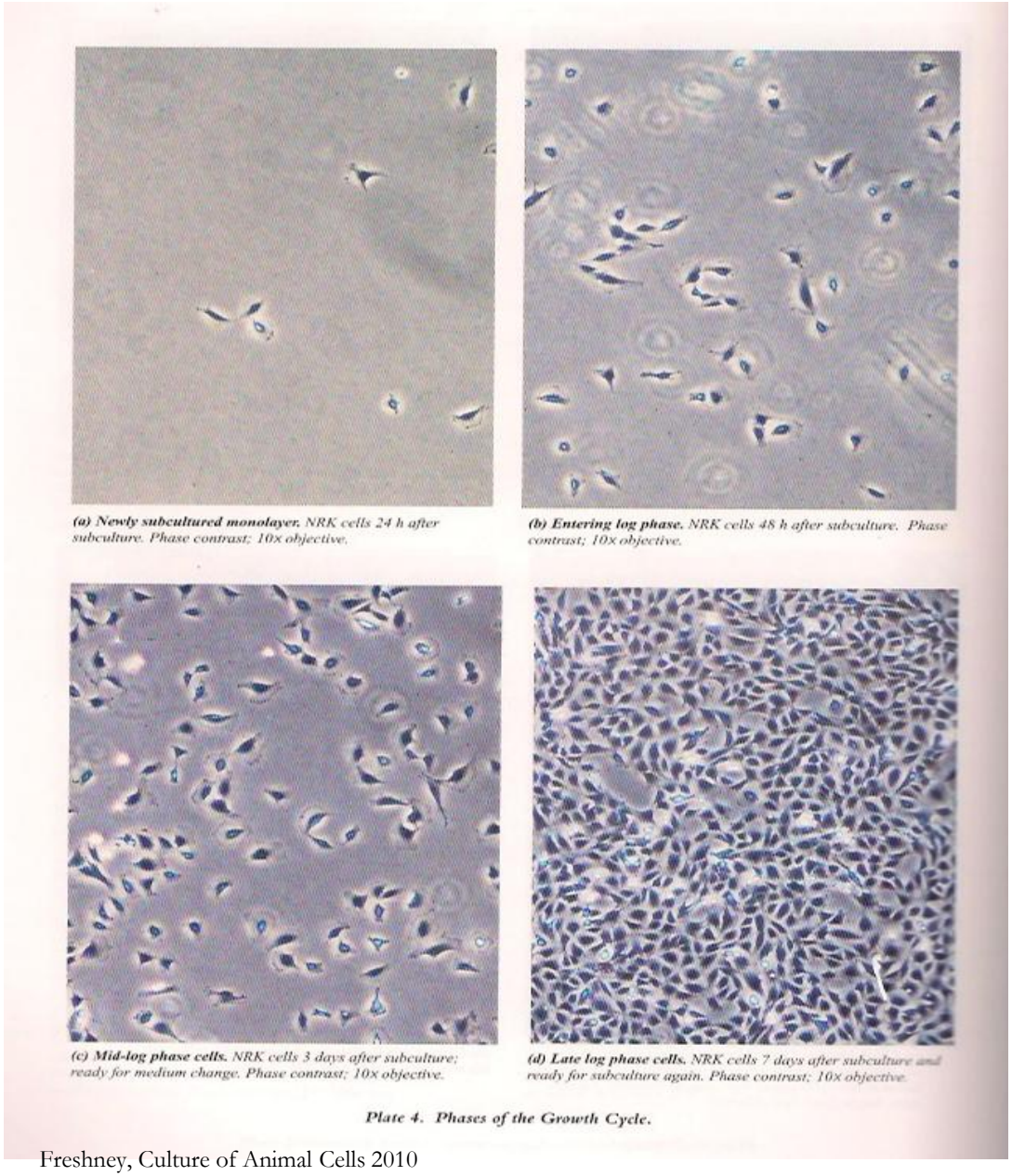
- Adherence to a strict schedule of subculture (e.g. change media after 4 days, subculture after 7 days) is best to assure reproducible behavior and monitoring

**(4) Requirements for other procedures**

- It is best to plan ahead for future experiments so that an adequate cell population is attained without disrupting normal cell cycling

# Phases of Life for a Subculture

Lag
Log
Plateau or Continue



***(a) Newly subcultured monolayer.*** *NRK cells 24 h after subculture. Phase contrast; 10× objective.*

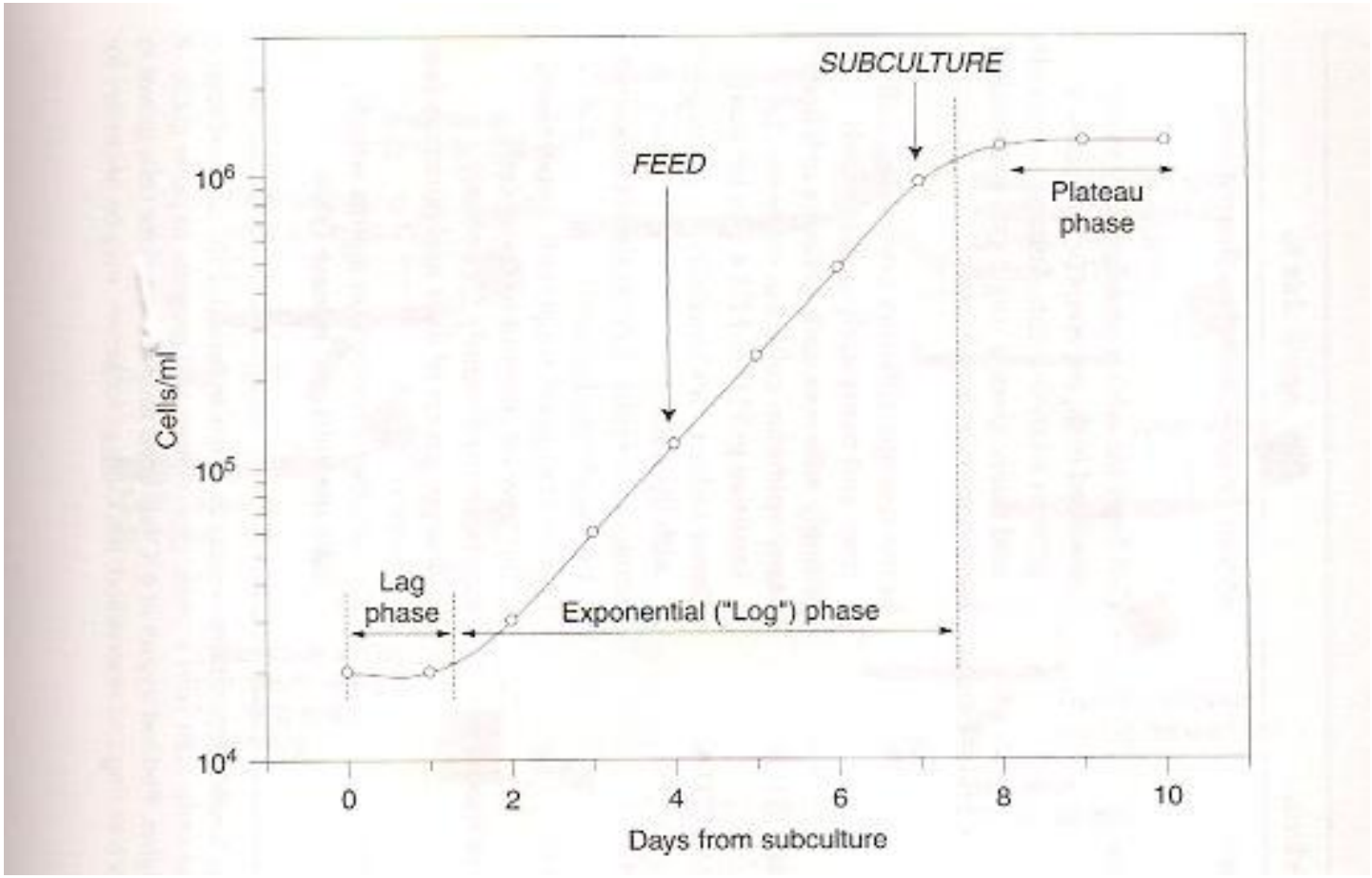
***(b) Entering log phase.*** *NRK cells 48 h after subculture. Phase contrast; 10× objective.*

***(c) Mid-log phase cells.*** *NRK cells 3 days after subculture; ready for medium change. Phase contrast; 10× objective.*

***(d) Late log phase cells.*** *NRK cells 7 days after subculture and ready for subculture again. Phase contrast; 10× objective.*

***Plate 4. Phases of the Growth Cycle.***

Freshney, Culture of Animal Cells 2010

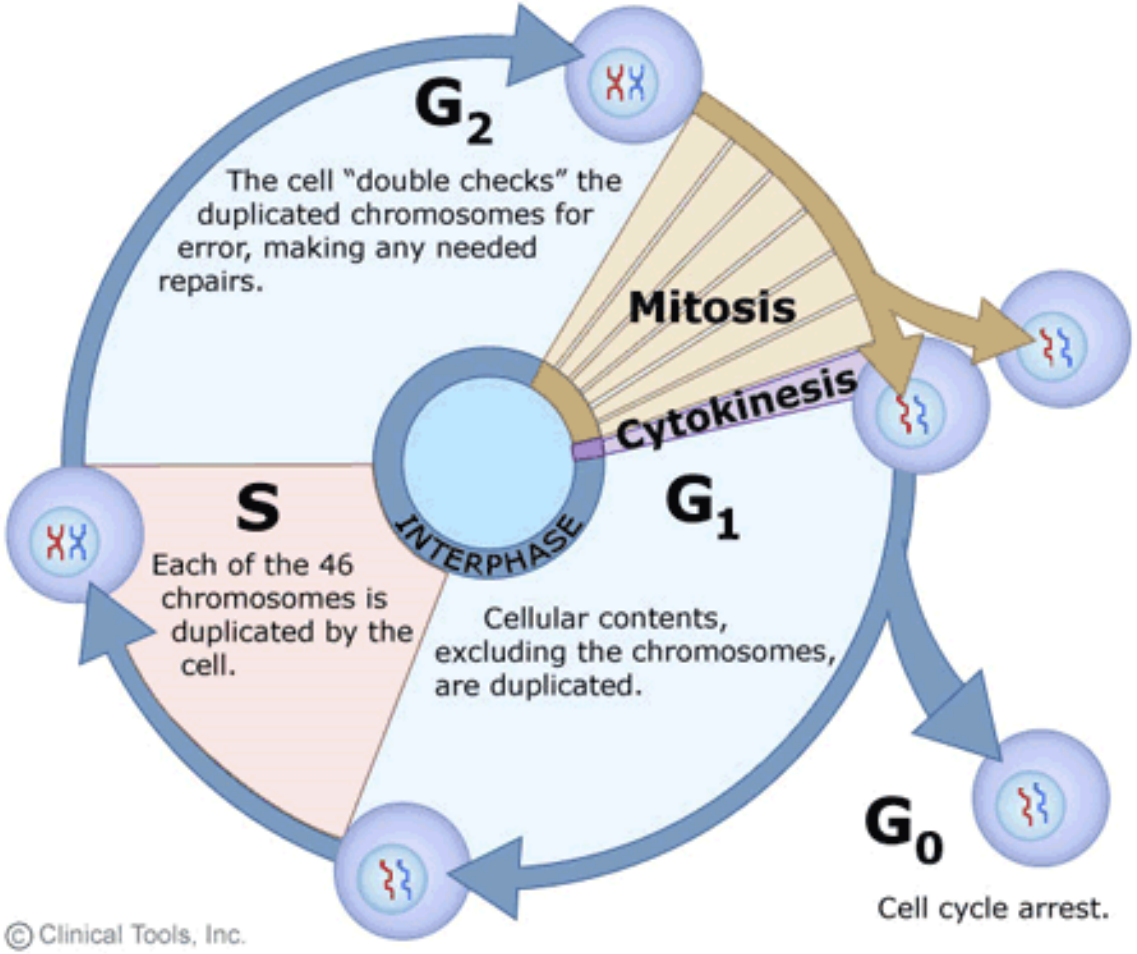



Freshney, Culture of Animal Cells 2010

# Ok… but, what's really going on??

**What stops the cells from growing?**
**Why do we even care if they stop growing?**
**Why does the media turn yellow?**
**How do I get the cells to detach so I can "split" them?**
**What effect would this have on my experiment?**

# Cell Cycle




For a normal cell the division cycle is a regulated and multistep process.

Recall: Telomerase and SV40 antigen

Cell cycle is governed by the microenvironment.

The cellular **microenvironment** is the immediate, small-scale environment in which the cells exist in a living organism, including blood vessels, immune cells, neighboring cells, signaling molecules and extracellular matrix.

# THE CELL CULTURE MICRO-ENVIRONMENT

1. Substrate
2. Contact (direct or indirect) with neighboring cells
3. The medium and gas phase

1. Extracellular matrix

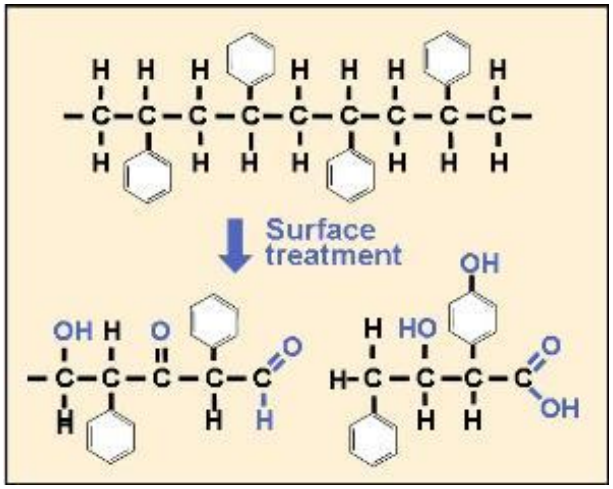
1. Cell adhesion molecules

# THE CELL CULTURE MICRO-ENVIRONMENT

## 1. Substrate

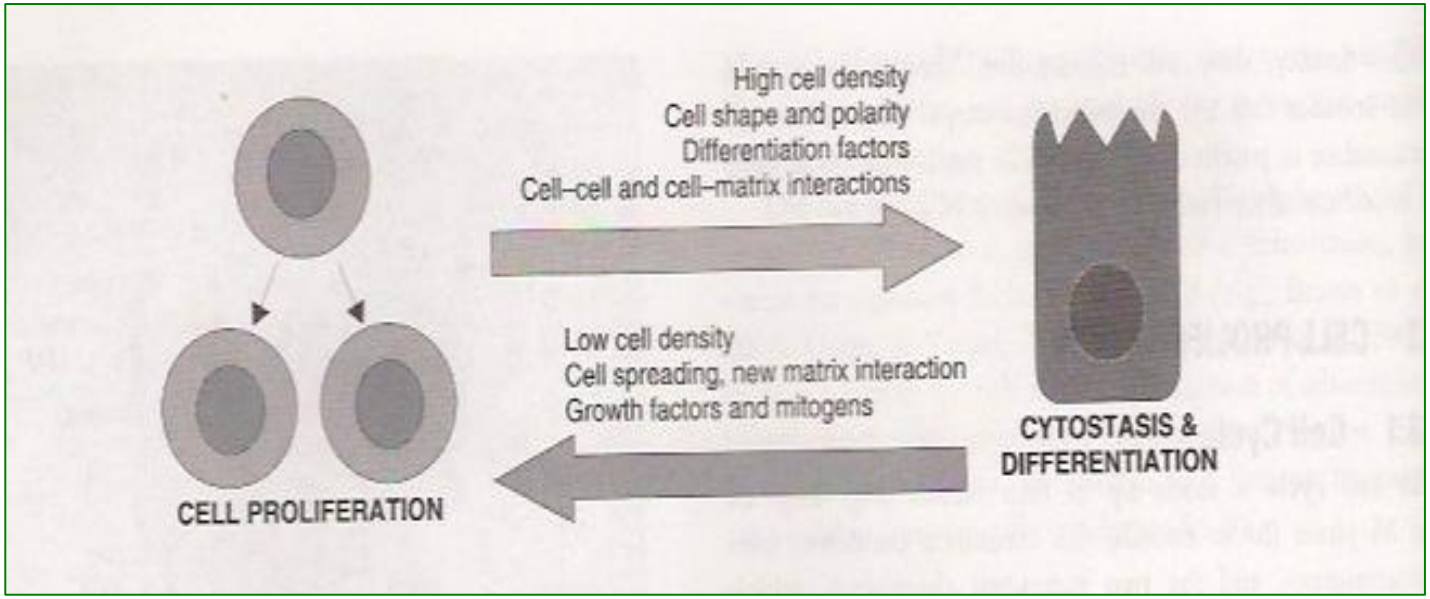
- Most cells from solid tissues grow as adherent monolayers and will need to attach to the substrate

- Culture flasks are made of a treated plastic (polystyrene) to create a slight net negative charge and hydrophilic surface



# THE CELL CULTURE MICRO-ENVIRONMENT

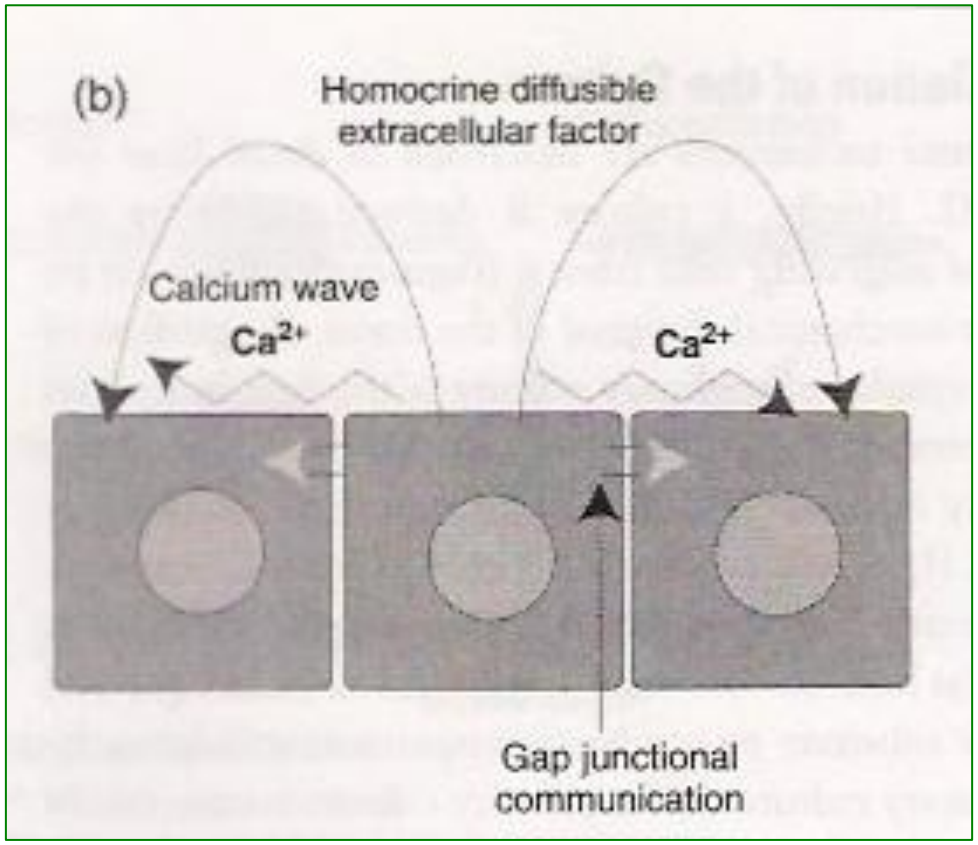
## 2. Contact (direct or indirect) with neighboring cells




Freshney, Culture of Animal Cells 2010

**What stops the cells from growing?**

**Why do we even care if they stop growing?**




Freshney, Culture of Animal Cells 2010

# THE CELL CULTURE MICRO-ENVIRONMENT

## 3. The medium and gas phase

- **neutral pH**
  - phenol red indicator
  - $CO_2$
  - buffer (bicarb, HEPES)
- **oxygen**
  - Risk of oxygen toxicity (free radicals)
  - medium depth
- **osmolality**
  - Human plasma is 290mOsm/kg
- **temperature**
  - Prefer body temperature 37°C
  - Consistency is vital
- **amino acids**
  - Essential amino acids
  - Glutamine is unstable and toxic
- **vitamins**
- **salts/electrolytes**
  - $-Ca^{2+}$ necessary for cell adhesion and cell signaling
  - $-Na^{+}$, $Cl^{-}$, $K^{+}$ regulate membrane potentials
  - -Primary contributors to osmolality
- **glucose**
- **growth factors**
  - Stimulate cell proliferation (mitogenic)
- **hormones**
  - Stimulate cell proliferation and adhesion
- **minerals**
- **antibiotics**

**Why does the media turn yellow?**

# THE CELL CULTURE MICRO-ENVIRONMENT

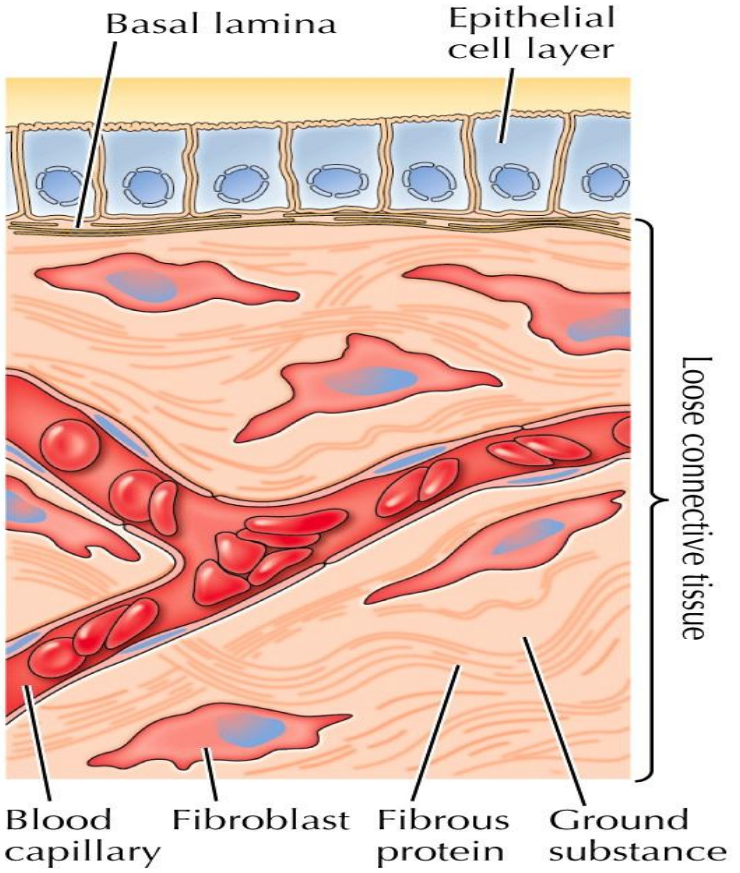
## 4. Extracellular matrix

- Secreted by the cells and mediates (a) cell adhesion to the substrate, as well as (b) cellular interactions
- Comprised variously of matrix structural proteins (collagen), polysaccharides (hyaluronan, proteoglycans), and adhesion proteins (fibronectin, laminin)
- For fastidious or specialized cells, substrates may be conditioned by previous cell growth or the addition of matrix constituents

**How do I get the cells to detach so I can "split" them?**

# THE CELL CULTURE MICRO-ENVIRONMENT

## 4. Extracellular matrix




***THE CELL: A MOLECULAR APPROACH 6e*, Figure 14.11**

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# THE CELL CULTURE MICRO-ENVIRONMENT

## 5. Cell adhesion molecules

Transmembrane proteins that mediate cell-cell interactions, cell-matrix interactions, and cell-substrate adhesions

**2 major classes of cell adhesion molecules:**

**(1) $Ca^{2+}$-dependent molecules**

**- cadherins**

cell-cell interactions

- selectins

unique to WBCs

**(2) $Ca^{2+}$-independent molecules**

- immunoglobulin superfamily

immune system molecules

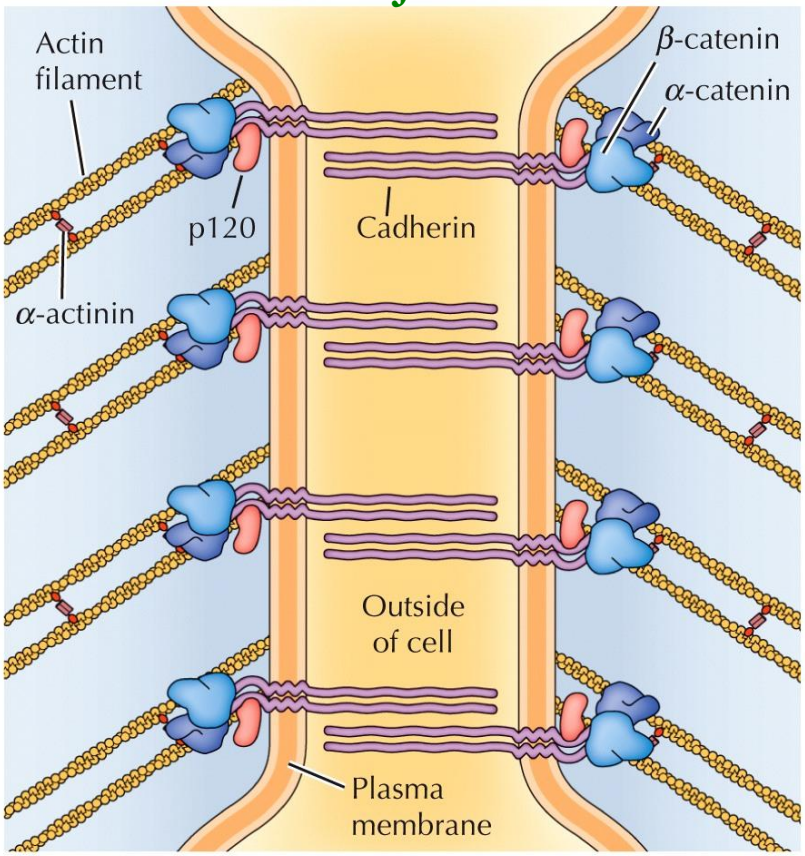
**- integrins**

cell-matrix interactions

# ADHESION JUNCTIONS

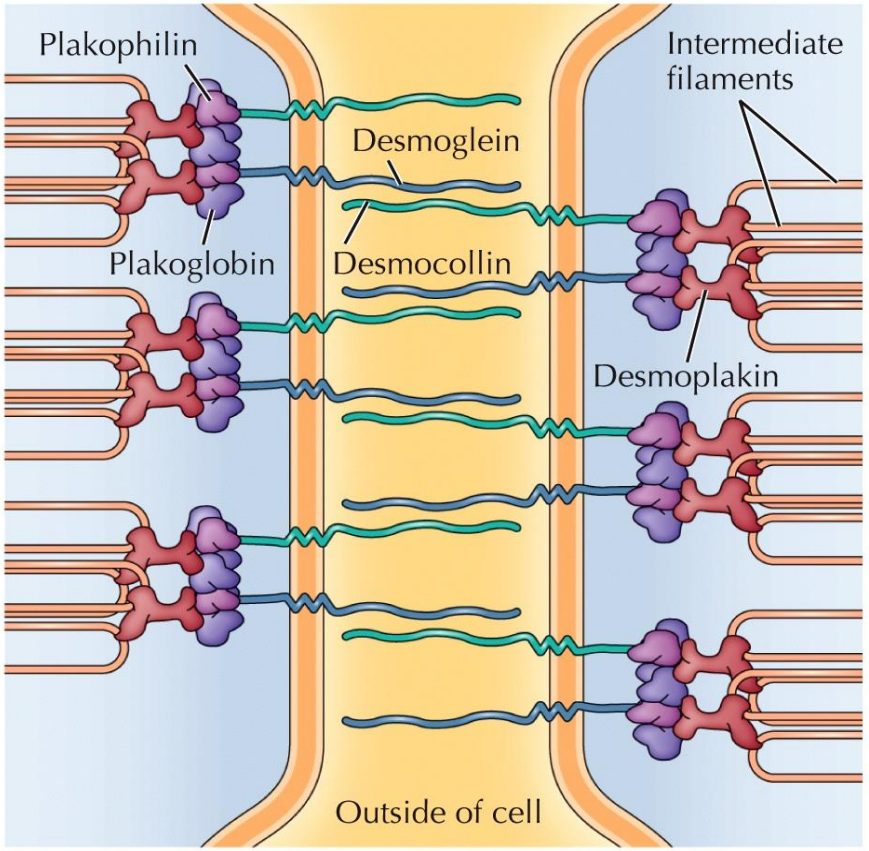
## cadherins

### Adherens Junctions




*THE CELL: A MOLECULAR APPROACH 6e*, Figure 14.24
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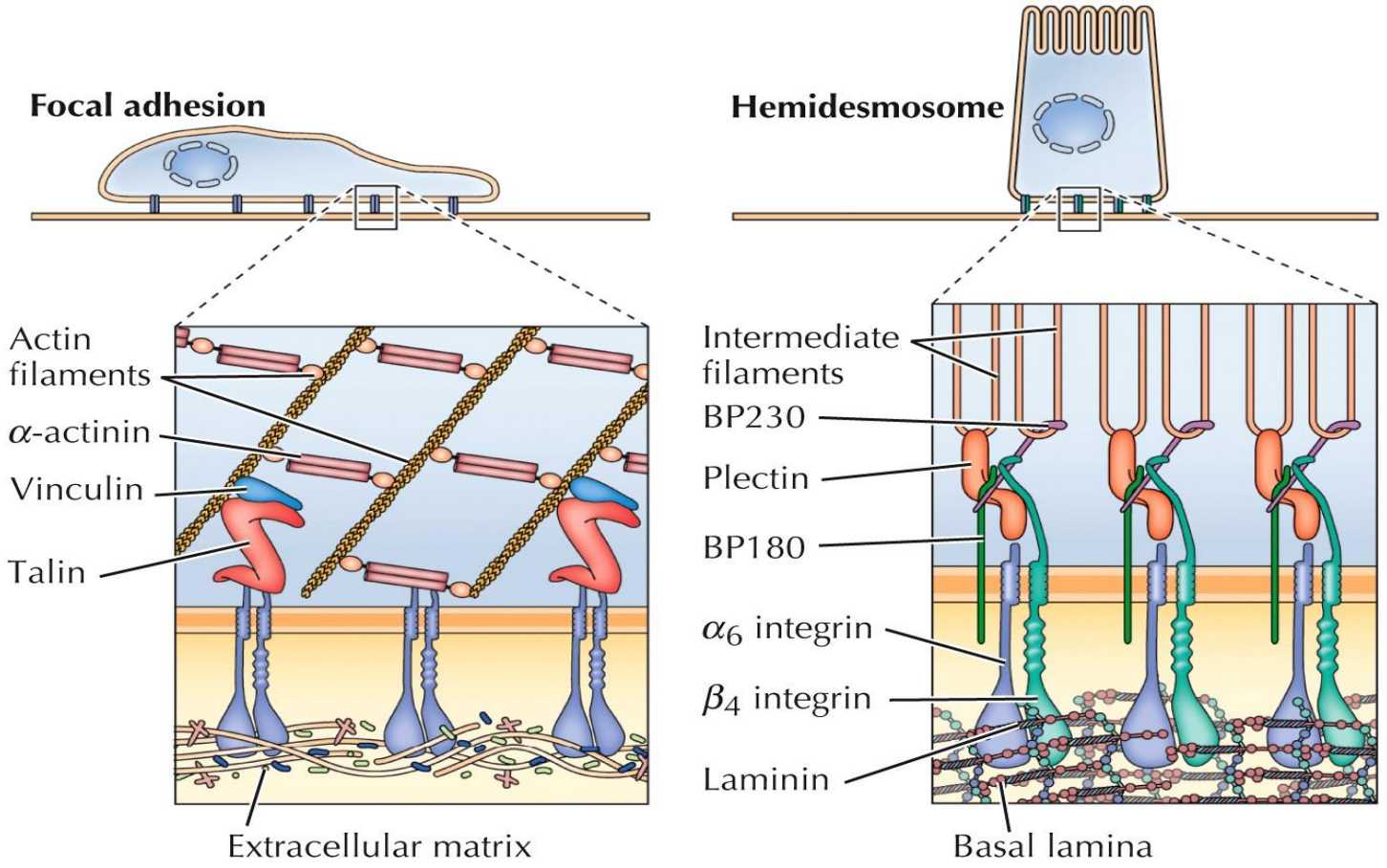
### Desmosomes




*THE CELL: A MOLECULAR APPROACH 6e*, Figure 14.25
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# ANCHORING JUNCTIONS

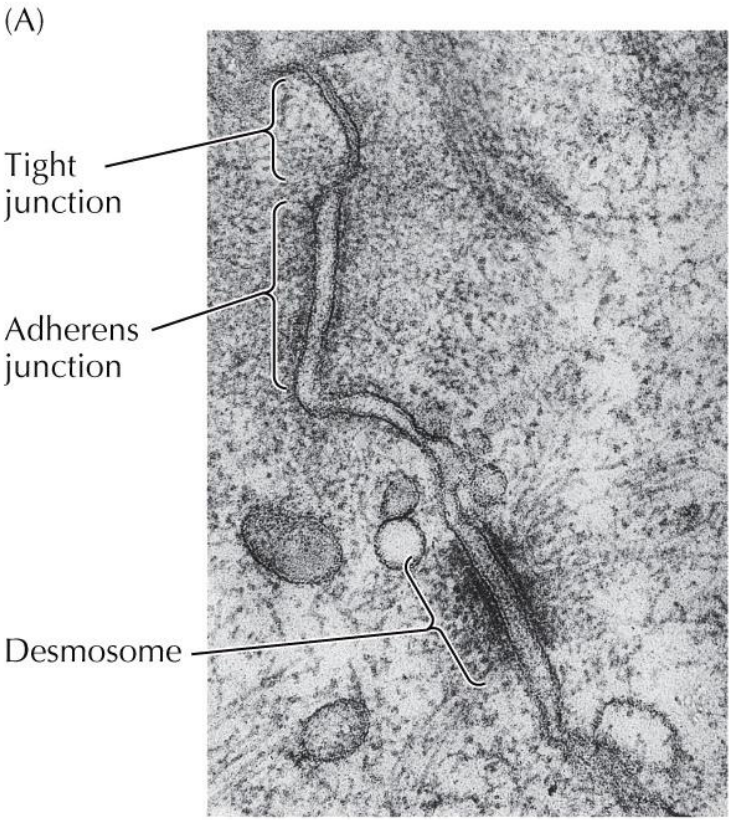
## Integrin

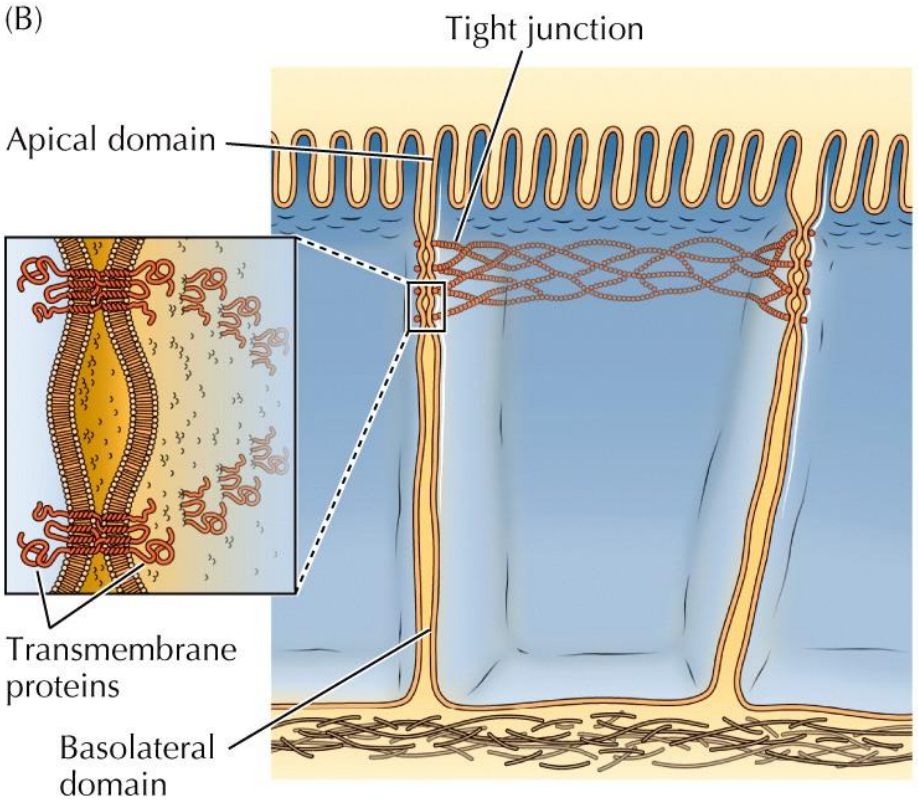



*THE CELL: A MOLECULAR APPROACH 6e*, Figure 14.21

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# TIGHT (OCCLUDING) JUNCTIONS




***THE CELL: A MOLECULAR APPROACH 6e*, Figure 14.26**

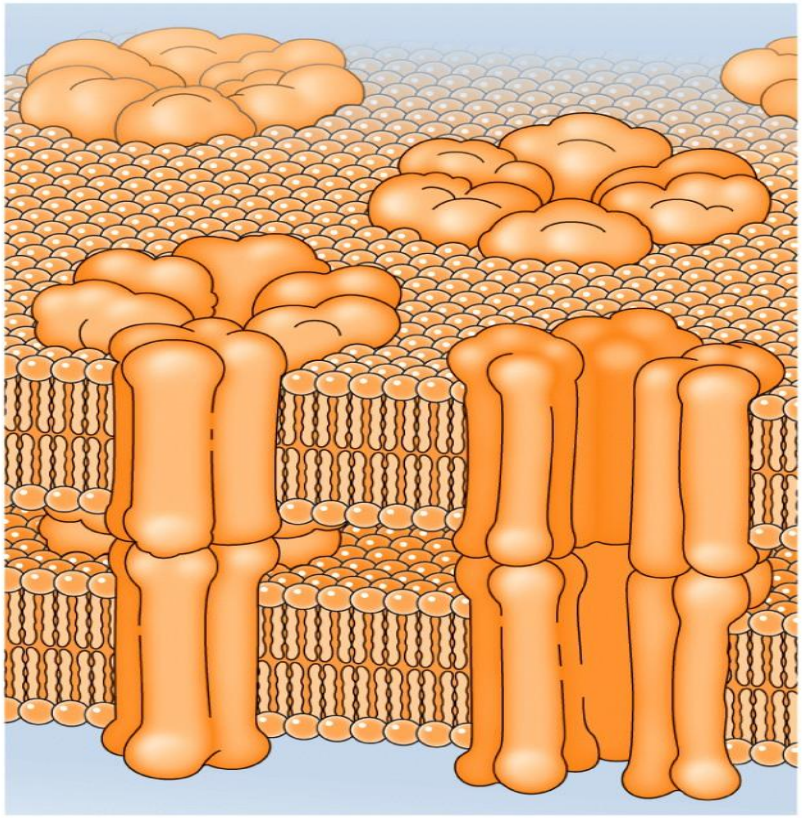
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# GAP (COMMUNICATING) JUNCTIONS

(A)



(B)



***THE CELL: A MOLECULAR APPROACH 6e*, Figure 14.28**

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# TRYPSIN

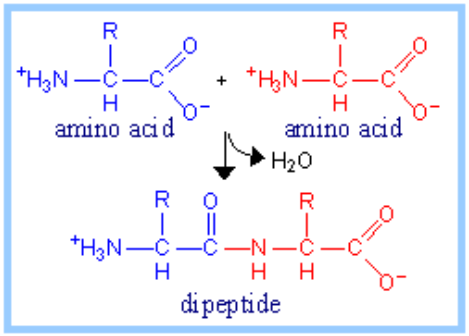
Trypsin is a protease which cleaves peptide bonds through hydrolysis

It is a normal enzyme in the digestive tract of all eukaryotes. Its precursor, trypsinogen, is secreted by the pancreas and activated in the duodenum (small intestine) to break down protein for absorption

** why isn't it in its active form in the pancreas??*

Ideal operating temperature is 37* and pH 7.5-8.5

Use caution not to expose cells longer than necessary






# By understanding the microenvironment… we understand **WHY** and **HOW** we subculture cells

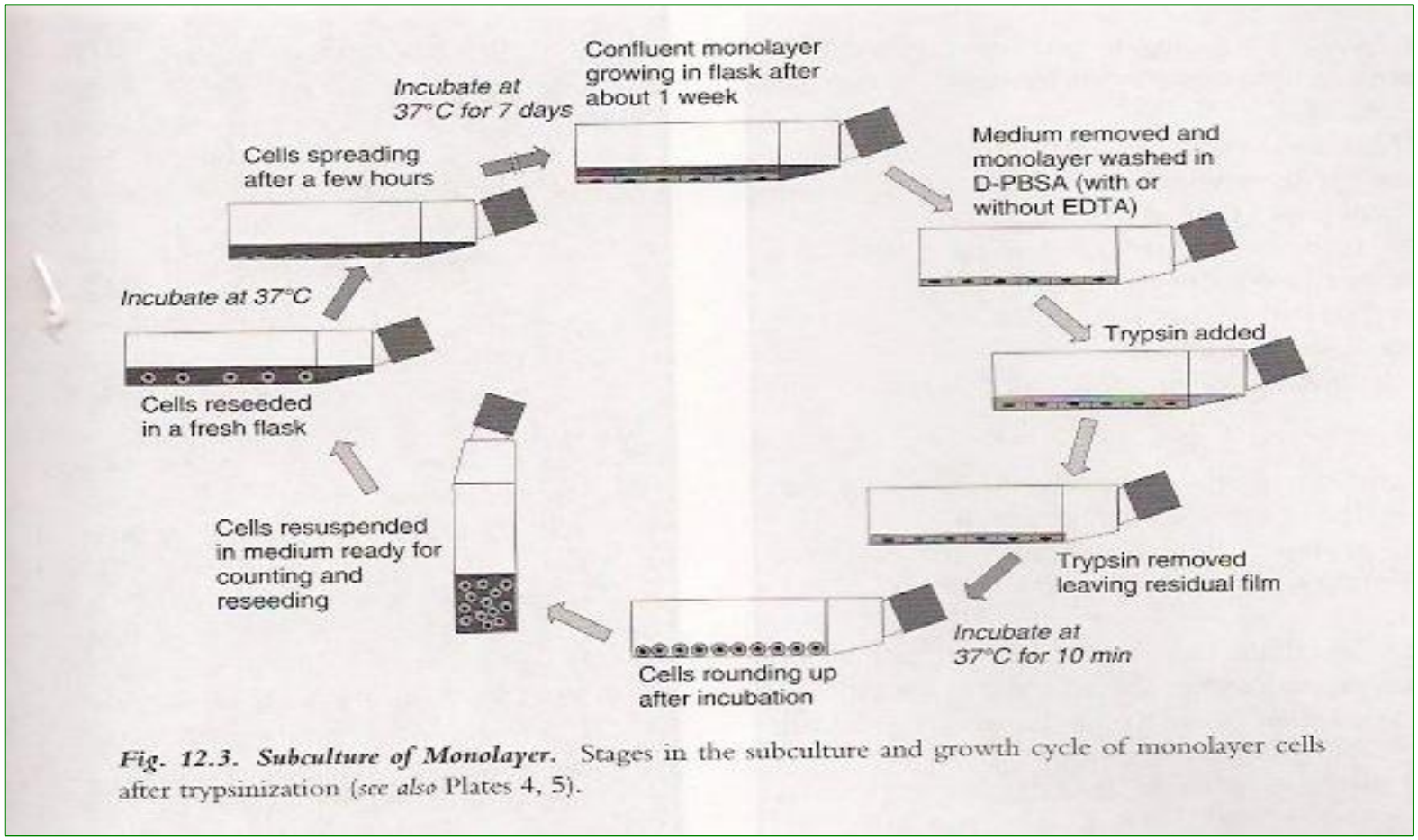
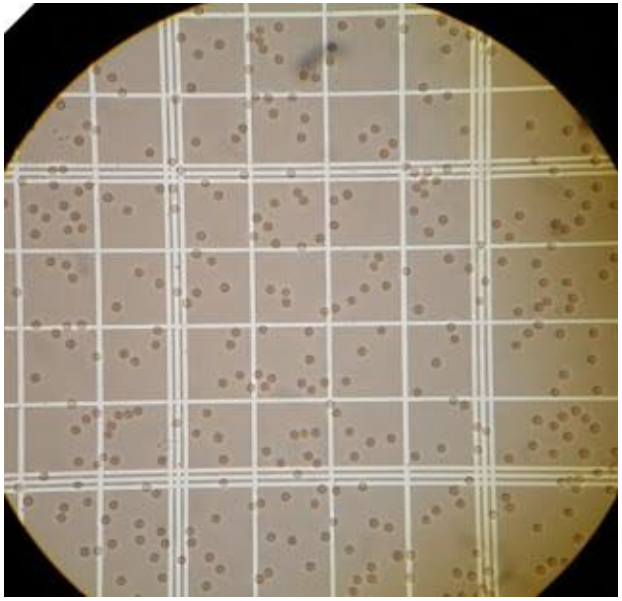



***Fig. 12.3. Subculture of Monolayer.*** Stages in the subculture and growth cycle of monolayer cells after trypsinization (*see also* Plates 4, 5).

Freshney, Culture of Animal Cells 2010

**Why do we "wash" the cells prior to trypsinizing??**



# Cell Quantitation

# Accuracy and Subculture

1) One flask evenly into several (1:5)

2) Estimate the percent confluence of a flask and determine how much you need for a lower percentage.

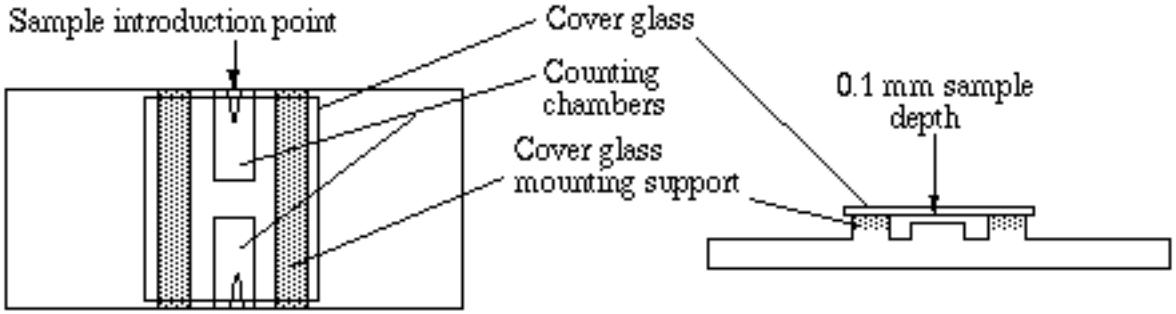
3) Count cell number and seed an exact cell number



# QUANTITATION OF CELLS

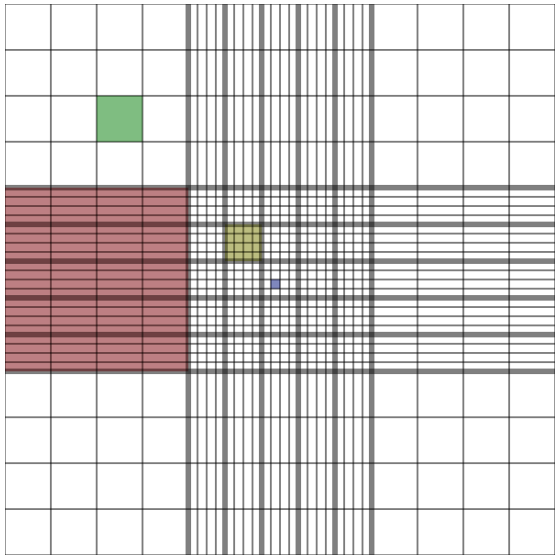
## hemocytometry





# QUANTITATION OF CELLS

## hemocytometry

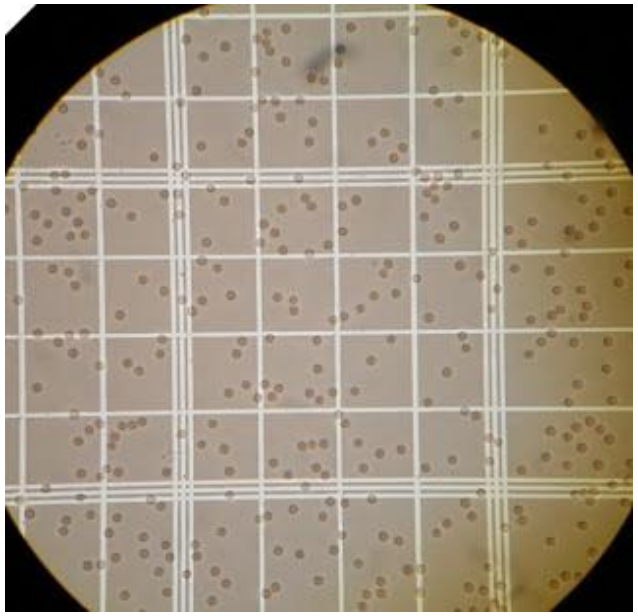


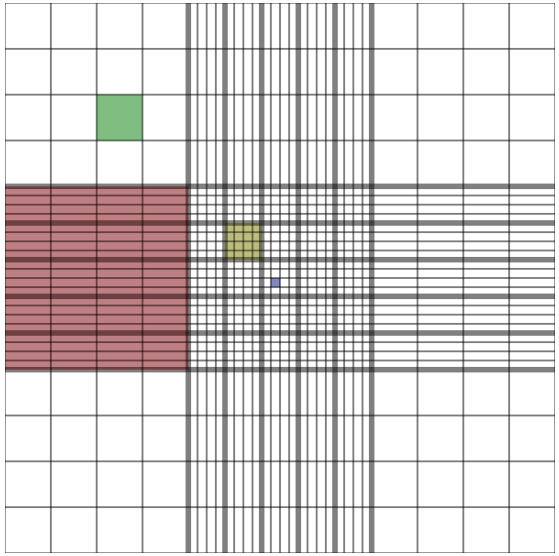
**Based on grid counts, you can calculate the number of cells/mL**

| Dimensions | Area | Volume (0.1mm depth) |
|---|---|---|
| 1 x 1 mm | $1mm^2$ | 100nL |
| 0.25 x 0.25 mm | $0.0625mm^2$ | 6.25nL |
| 0.2 x 0.2 mm | $0.04mm^2$ | 4nL |
| 0.05 x 0.05 mm | $0.0025mm^2$ | 0.25nL |

# QUANTITATION OF CELLS

## hemocytometry





S1 = 56
S2 = 62
S3 = 44
S4 = 65
S5 = 51

When counting 5 yellow squares

Cells/nl = (n x 5)/100nl

To convert to ml multiply by $10^6$

# Determine Seeding Density

You know how to count and how to get a certain percentage of confluence when splitting into the same size flask.

For experiments, you will often need to split your cells from a flask to a multiwell plate.

Calculate the difference in surface area to determine what to seed.



| Plate | Surface Area ($mm^2$) | Seeding Density | Growth Medium (ml) |
|---|---|---|---|
| 24-well | 200 | $0.05 \times 10^6$ | 0.5-1.0 |
| 12-well | 401 | $0.1 \times 10^6$ | 1-2 |
| 6-well | 962 | $0.3 \times 10^6$ | 3-5 |
| 60mm | 2827 | $0.8 \times 10^6$ | 5 |
| 100mm | 7854 | $2.2 \times 10^6$ | 10 |