



JOHNS HOPKINS

WHITING SCHOOL  
of ENGINEERING

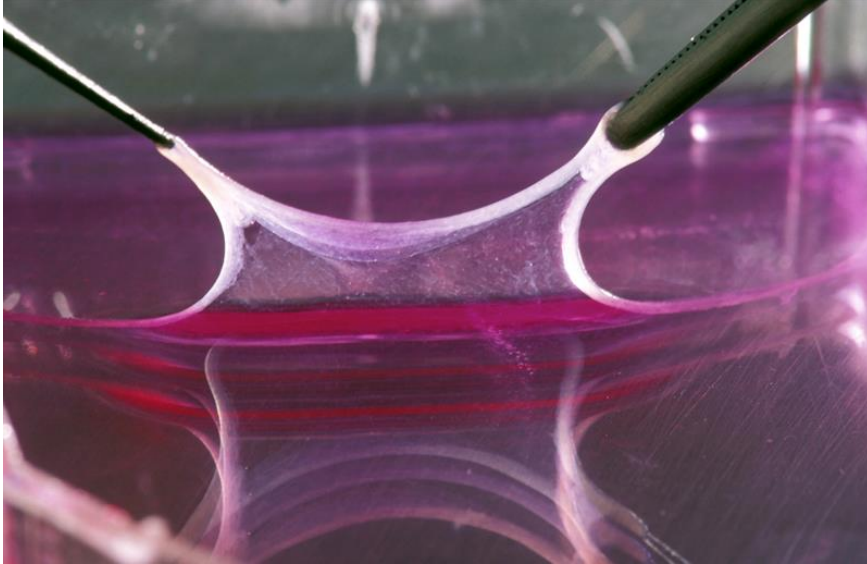
# Cell and Tissue Engineering

Cell Numbers, Growth and Kinetics

Part 1 Cell Numbers

# What are clinically meaningful cell numbers?

HEALs – Human Ectopic Artificial Livers



**How many cells do I need in the end?**

**How many cells do I need to start with?**

**How fast can I grow tissue?**

# What are meaningful cell numbers?

Density of cells in the human body  $\sim$  1-3 billion per milliliter ( $1\text{cm}^3$ )

1 Milliliter

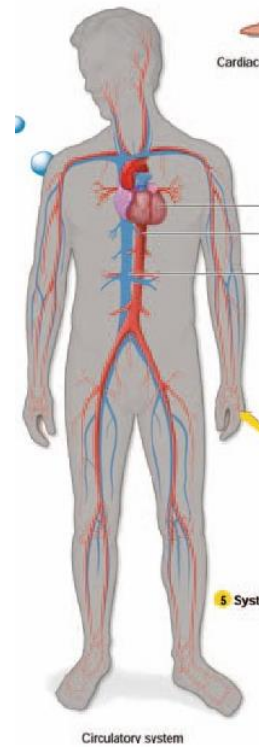




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Volume of 70kg ( $\sim 150\text{lb}$ ) human  $\sim 70,000\text{ml}$



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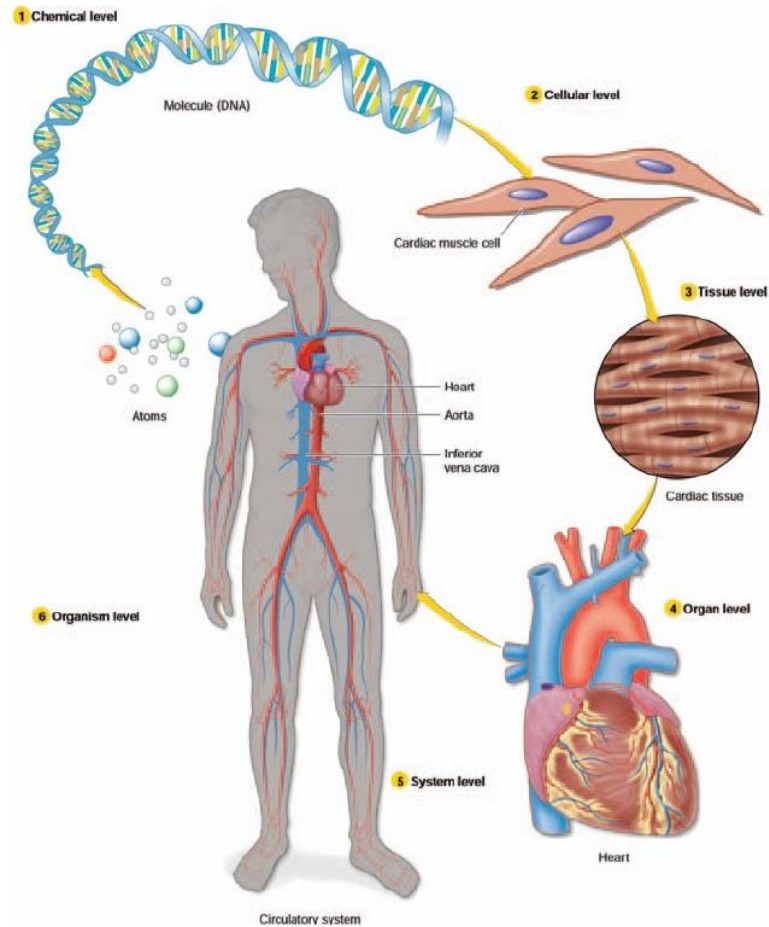


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***Number of cells in the human body  $\sim 100$  trillion ( $10^{14}$ ) cells***



1 Milliliter

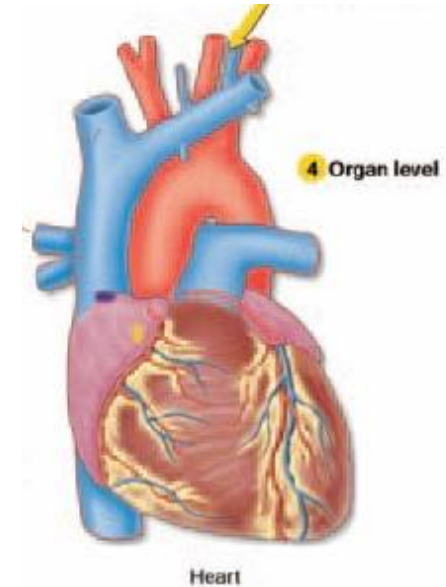
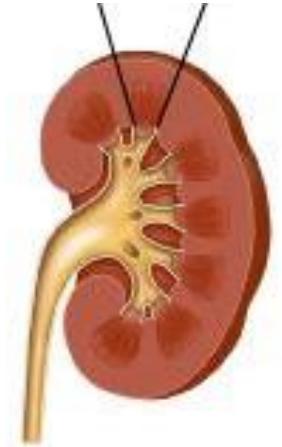


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Volume of an average organ  $\sim$  100-500ml

***Cells in an average organ  $\sim$  100-1500 ( $10^9$ - $10^{11}$ ) billion cells***



# What are meaningful cell numbers?

Volume of an average organ  $\sim 100\text{-}500\text{ml}$

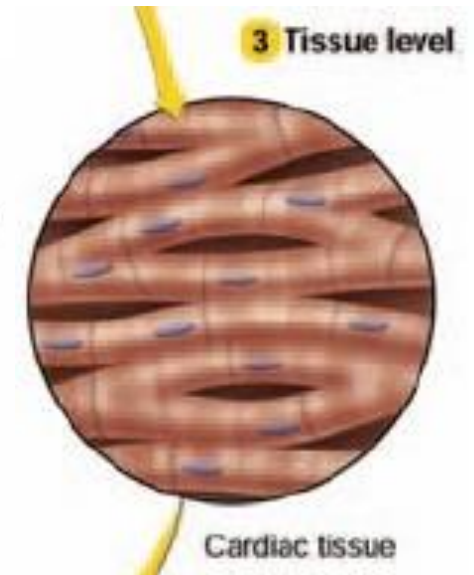
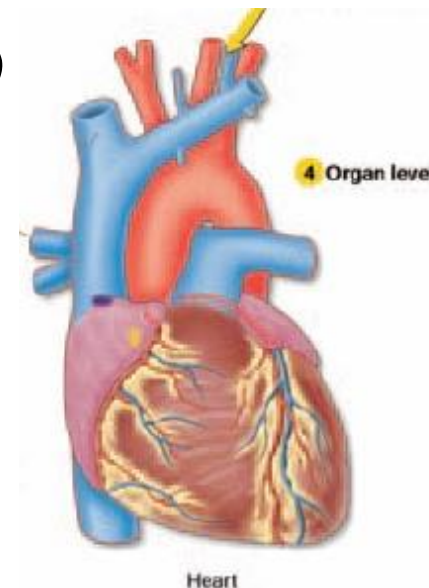
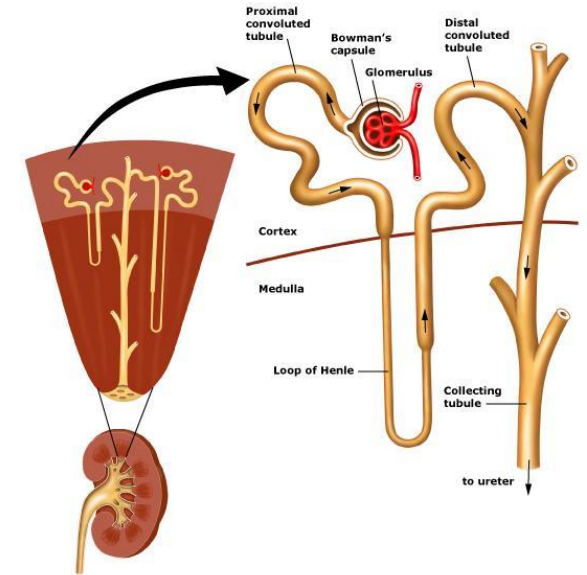
***Cells in an average organ  $\sim 100\text{-}1500$  ( $10^9\text{-}10^{11}$ ) billion cells***

Volume of a function subunit  $\sim 100\mu\text{m}^3$

***Cells in a functional subunit  $\sim 500\text{-}1000$  ( $10^2\text{-}10^3$ )***

(recall cells on on the order of  $10\mu\text{m}$ )

(mixed populations!)



# What are CLINICALLY meaningful cell numbers?



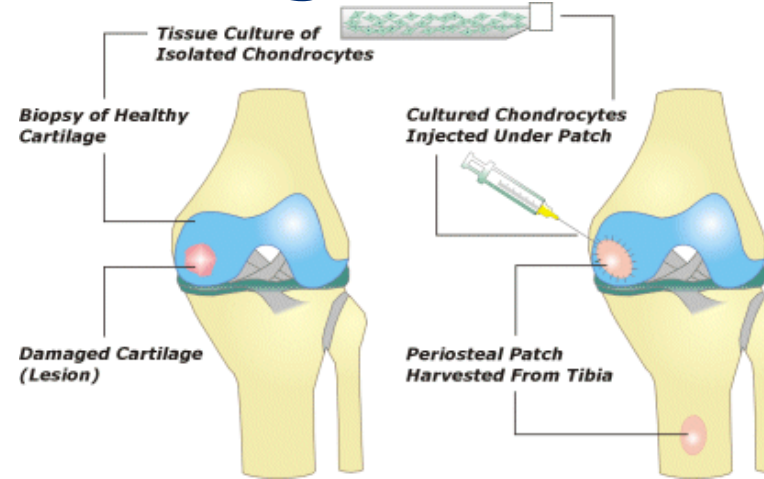
Whole body	$\sim 10^{14}$
Whole organ	$\sim 10^9 - 10^{11}$
Functional Unit	$\sim 10^2 - 10^3$

***Typical cellular therapies require  $10^7 - 10^9$  cells***



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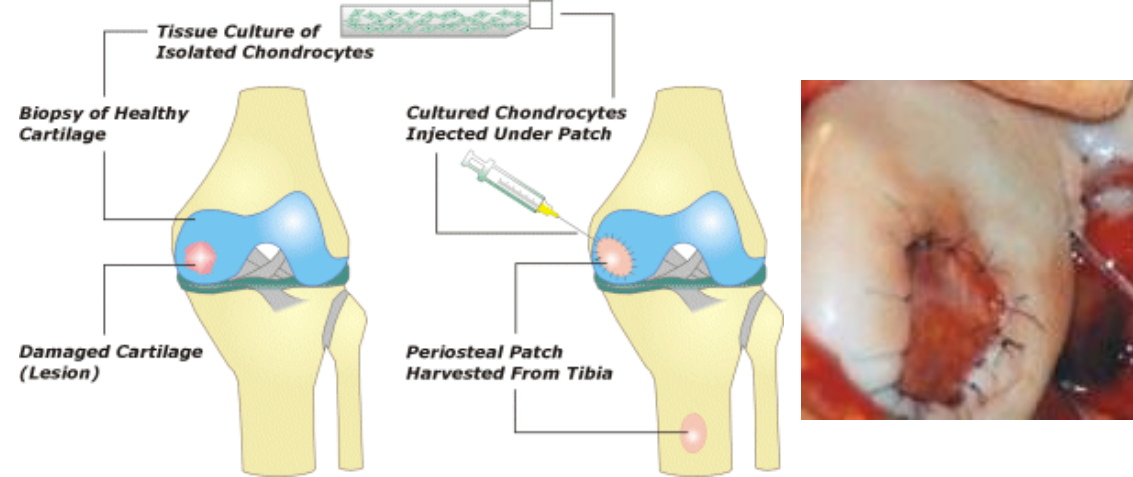


## ***Typical cellular therapies require $10^7 - 10^9$ cells***

Chondrocyte transplants	$\sim 10^7$
Lymphocyte therapies	$\sim 10^9$
Bone marrow transplant	$\sim 10^9$
Liver support	$\sim 10^{10}$
Skin grafts	$\sim 10^7$ per patch
	x many per ulcer!

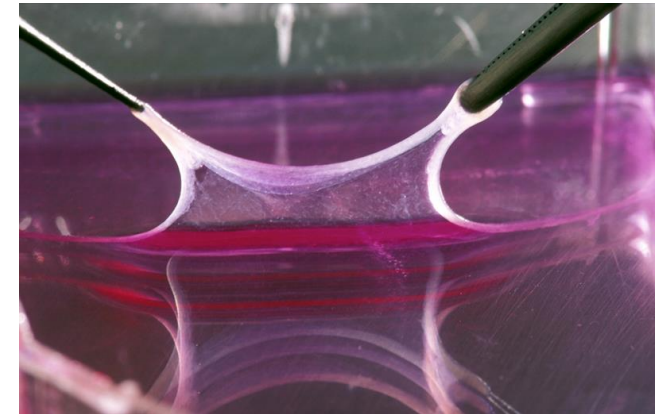
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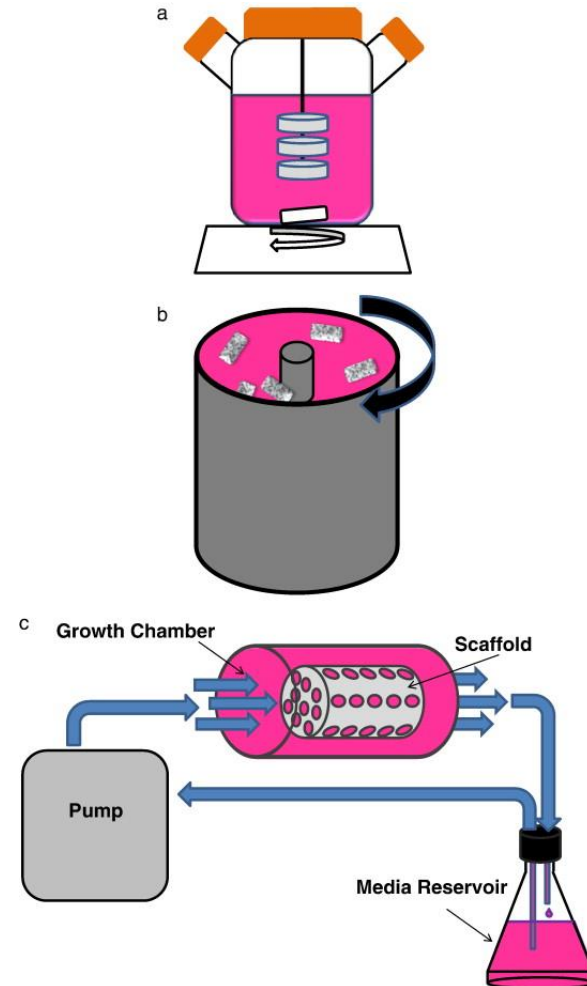
# What are CLINICALLY meaningful cell numbers?

Tissue-like cultures require  $10^6$  cells per milliliter – **NOT the billions of cells per milliliter that your body houses!**

Therefore, bioreactors need to be on the order of 100's of milliliters in volume

X many (they are single use)

X disposable production technology

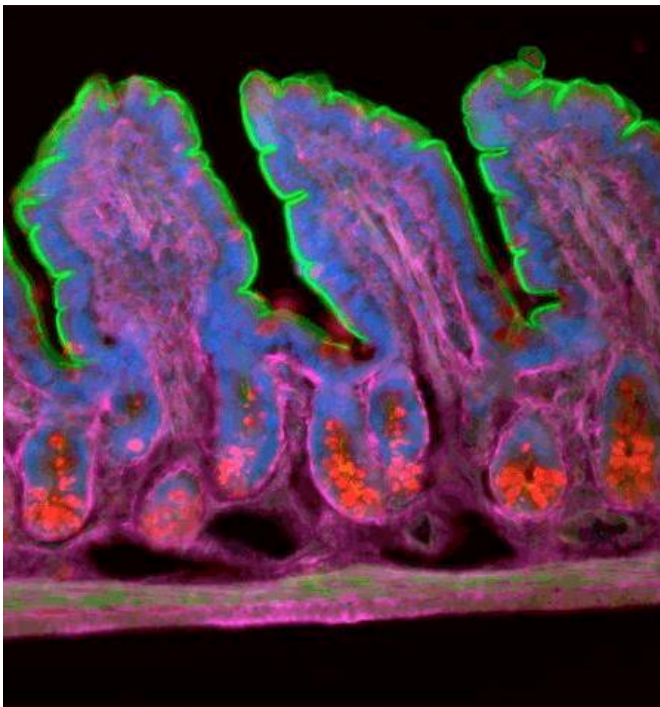


# How do we get from 1 cell embryo to a human?

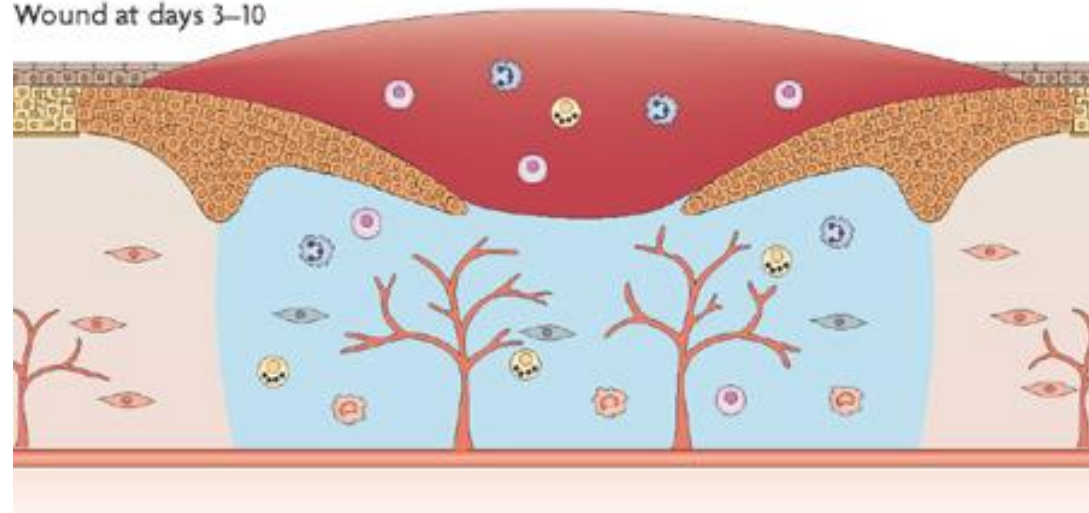




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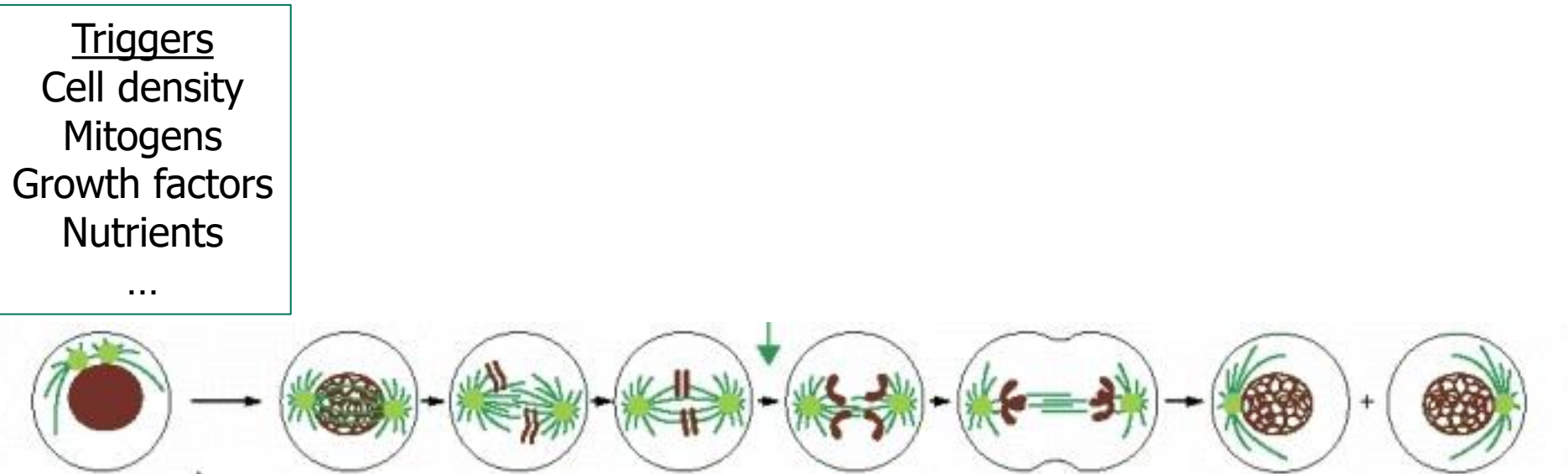


Wound at days 3–10



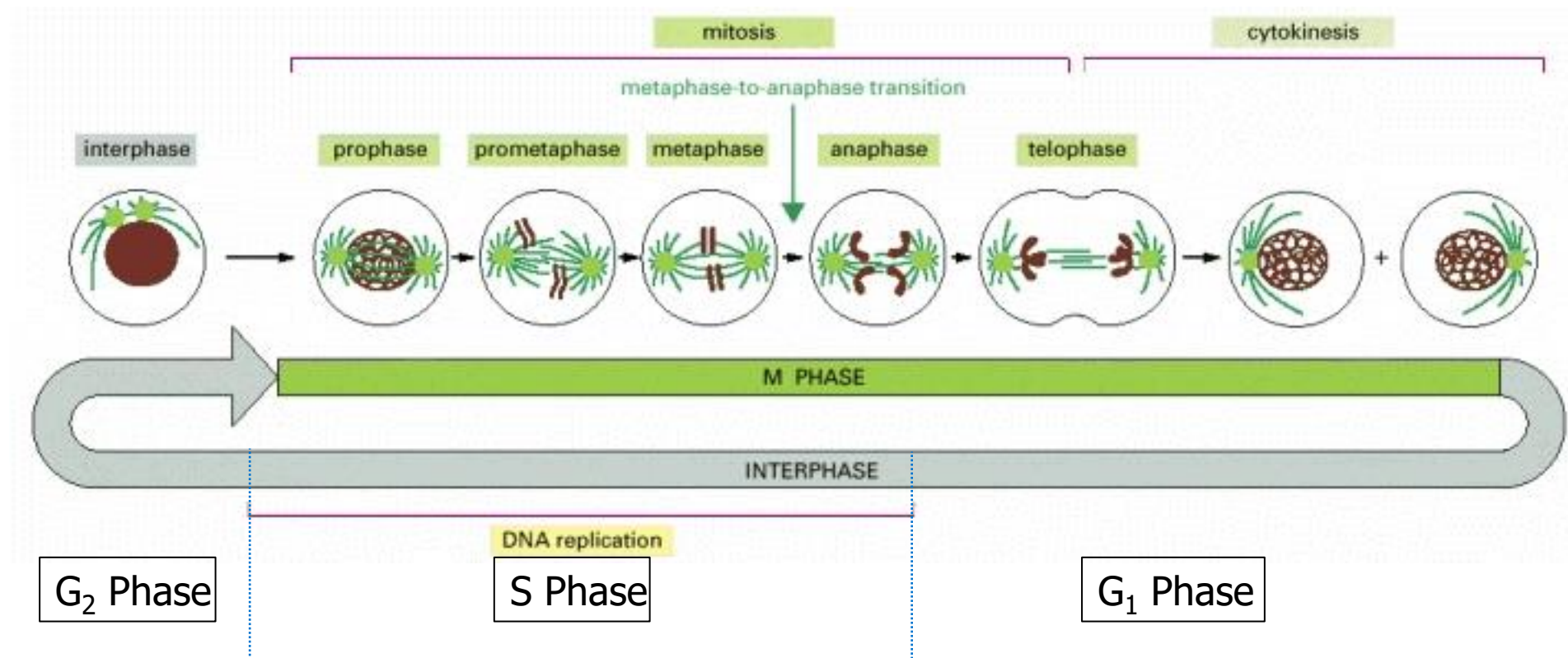
# How does cell division work?

Here's what eukaryotic cell division looks like under a microscope... (as a cartoon!)



# How does replication work?

M phase <1hr  
Interphase ~24hr



# Replication checks and balances

## **G<sub>1</sub> and G<sub>2</sub> gaps & checkpoints**

*Is everything going well?*

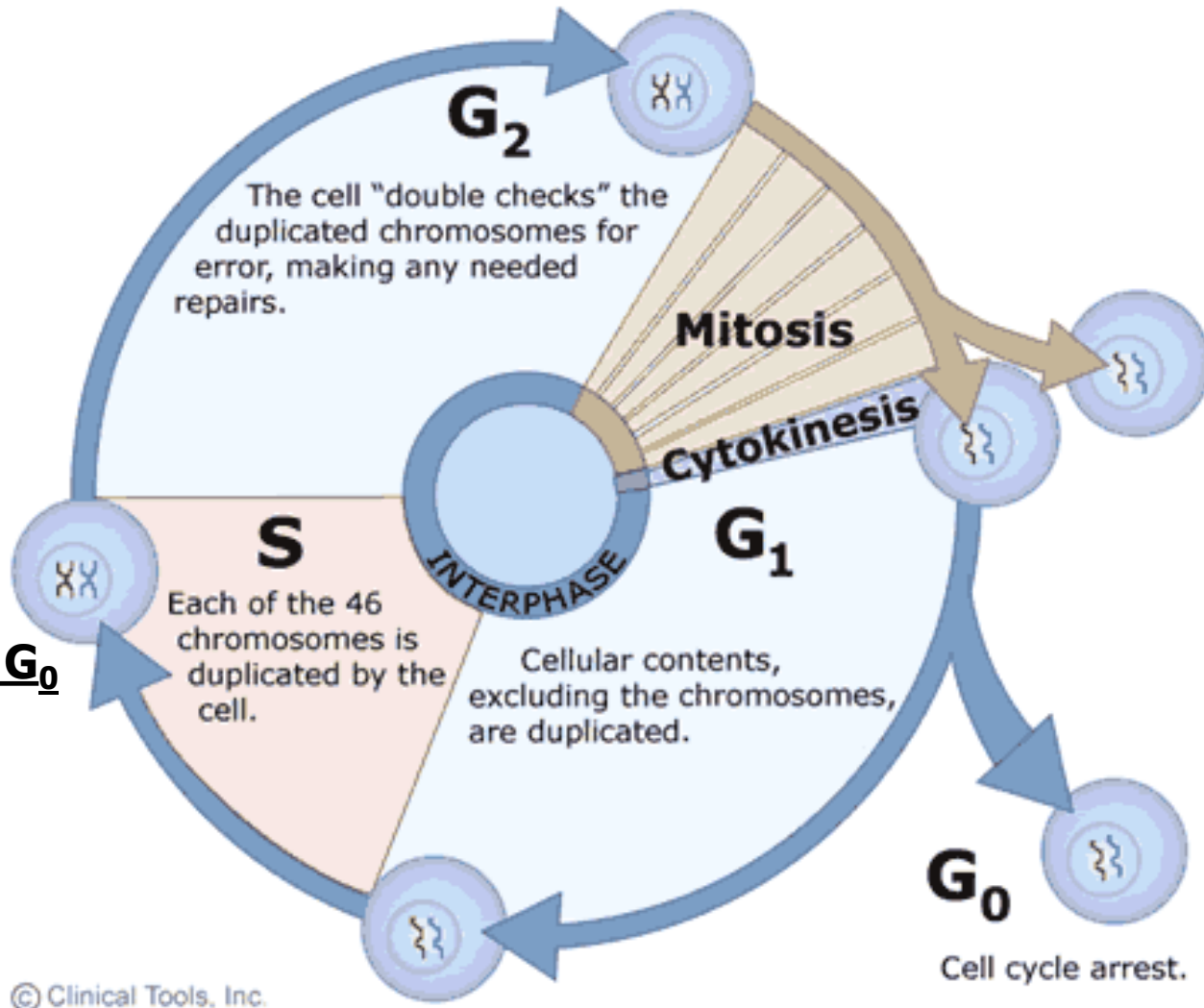
## **Terminally Quiescent in G<sub>0</sub>**

Neurons

Fat cells

Hair cells of the inner ear

Cardiac cells





# Replication checks and balances

$^{14}\text{C}$  dating



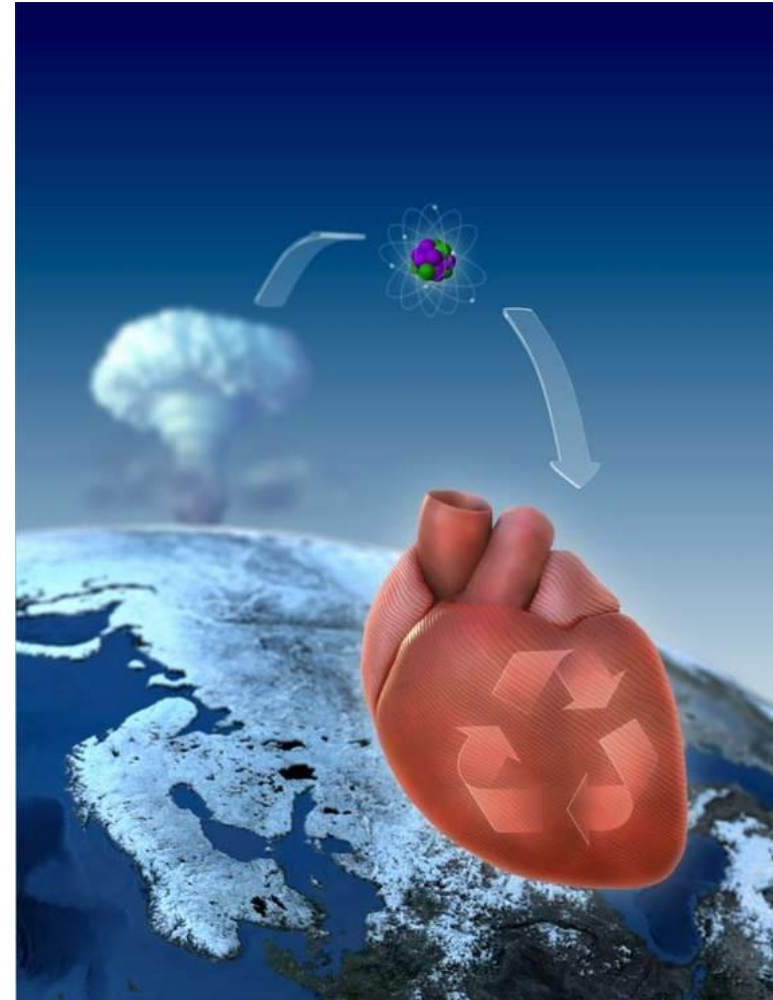
## Terminally Quiescent in $G_0$

Neurons

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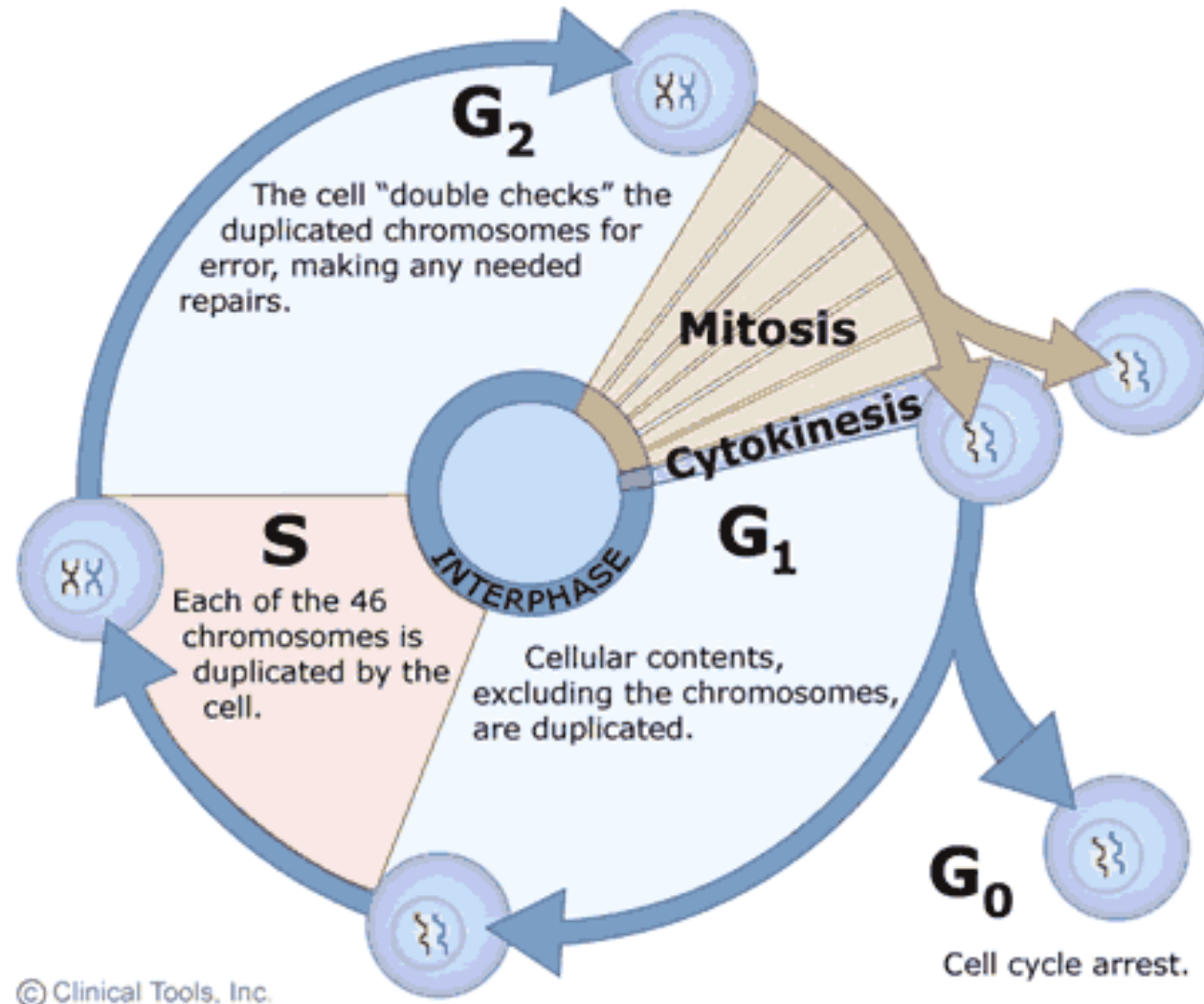
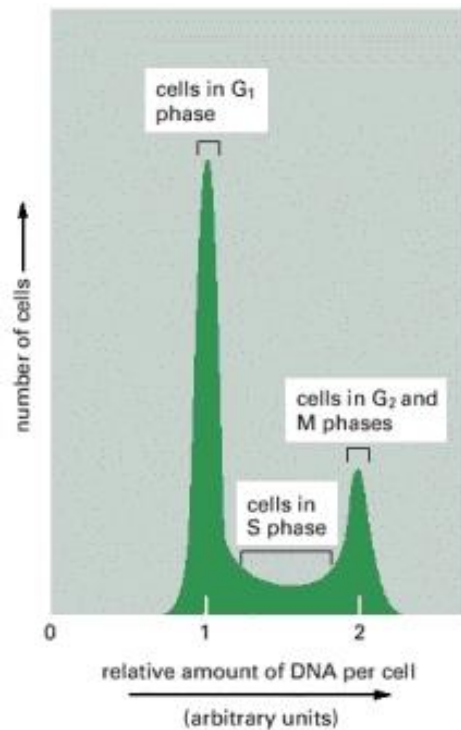
Hair cells of the inner ear

~~***Cardiac cells??***~~

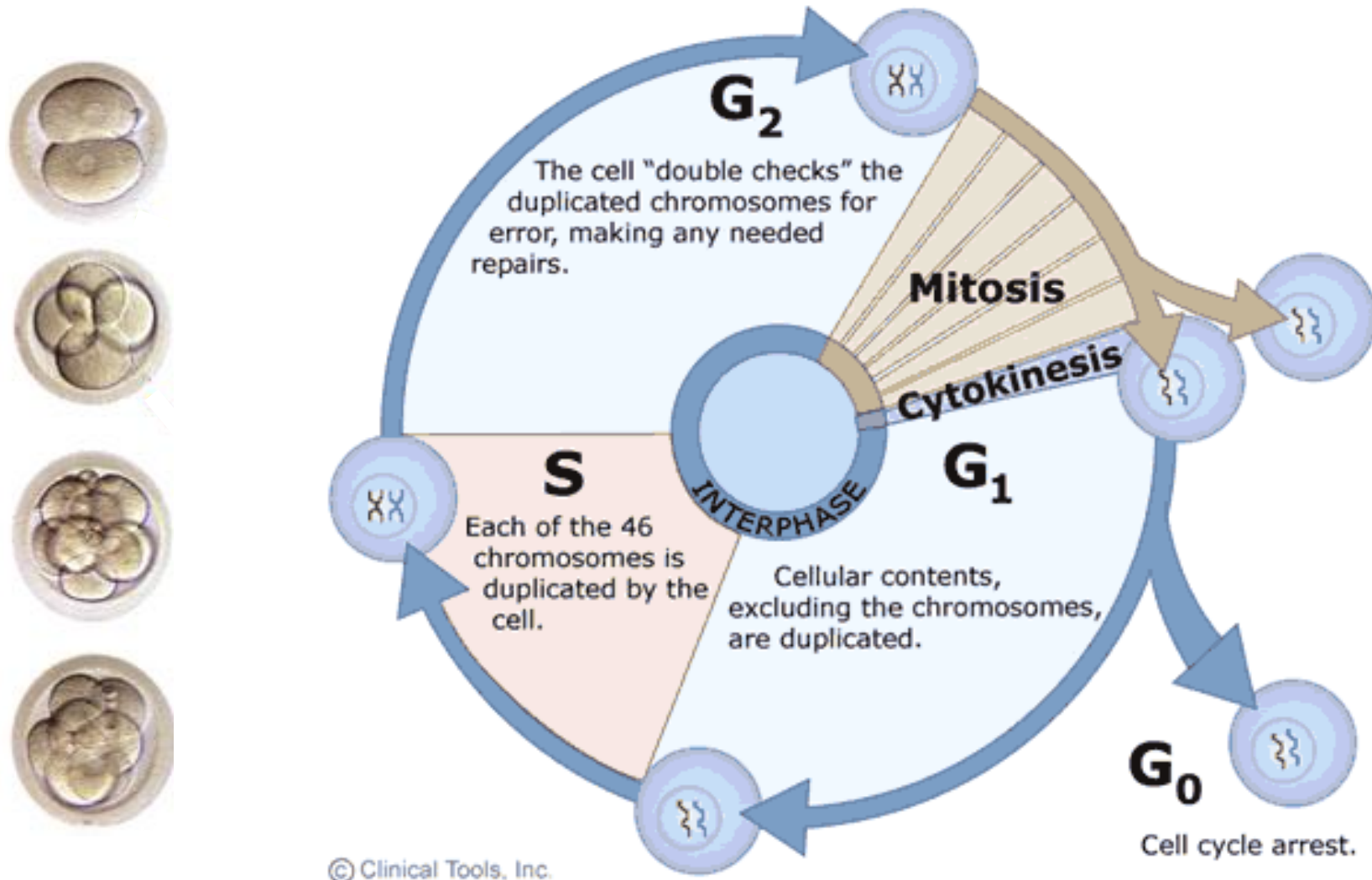


# Replication check and balances

## $G_1$ and $G_2$ gaps & checkpoints.



# Replication check and balances

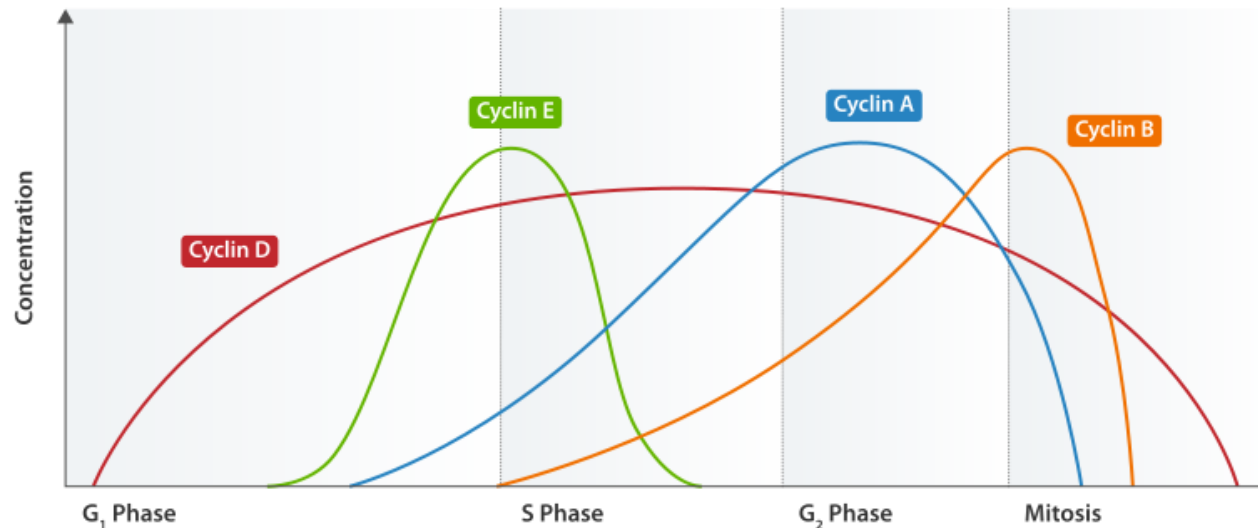


# How is cell division regulated?

## Cyclins

**CDKs** – cyclin dependent kinases

- Require a **cyclin** molecule to be activated
- Trigger passage between phases of the cell cycle
- Regulated by activation/deactivation, production/destruction



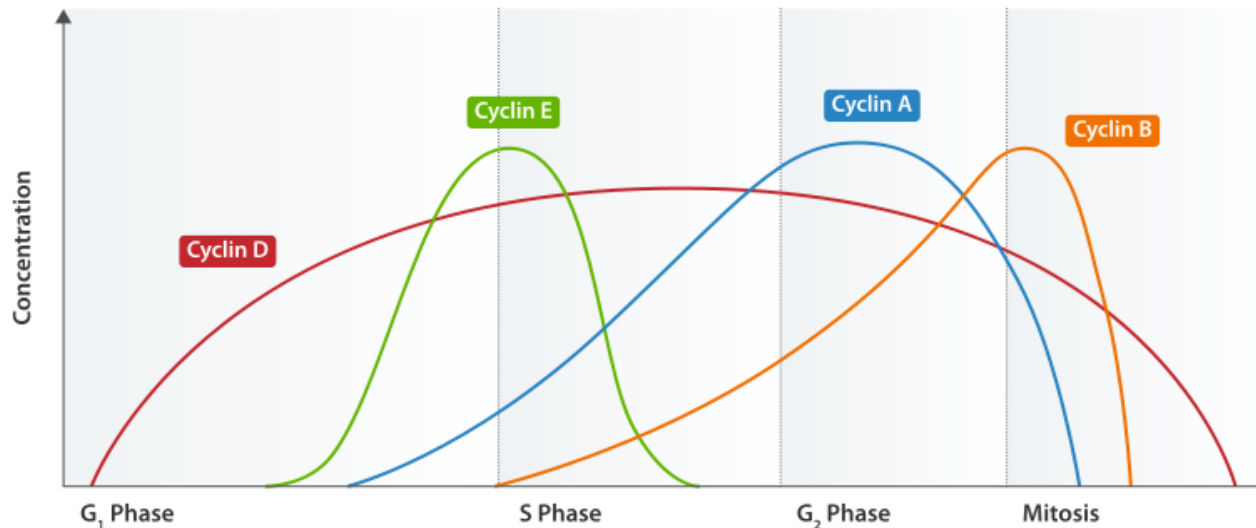


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### CDKs – cyclin dependent kinases

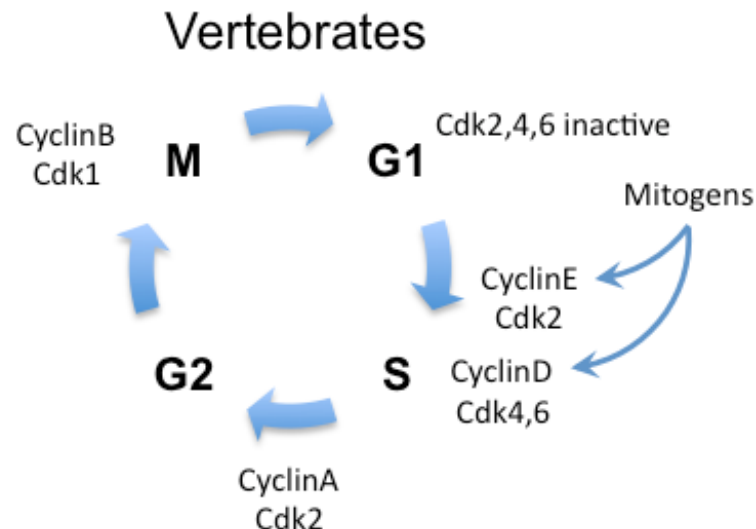
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*"By the way the name cyclin, which I coined, was really a joke, it's because I liked cycling so much at the time but they did come and go in the cell..."*

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# The other side of the coin: apoptosis

*Programmed Cell Death*

Human (tissue/organ) development requires apoptosis!

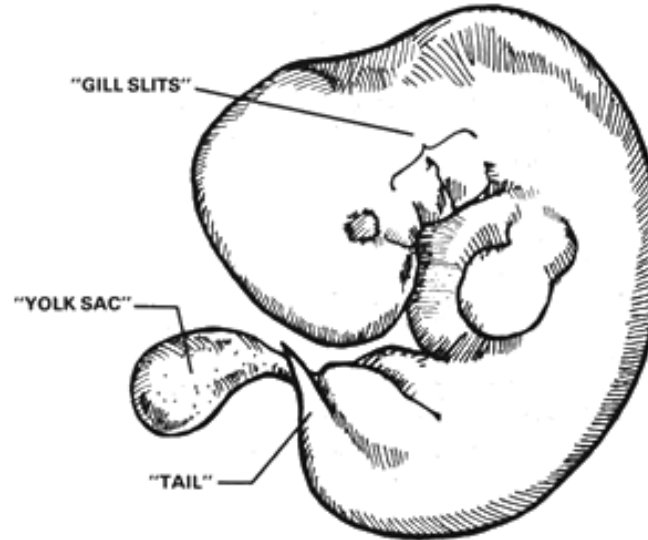
Natural morphogenesis (and healing) over produces materials that are later pruned back

# The other side of the coin: apoptosis

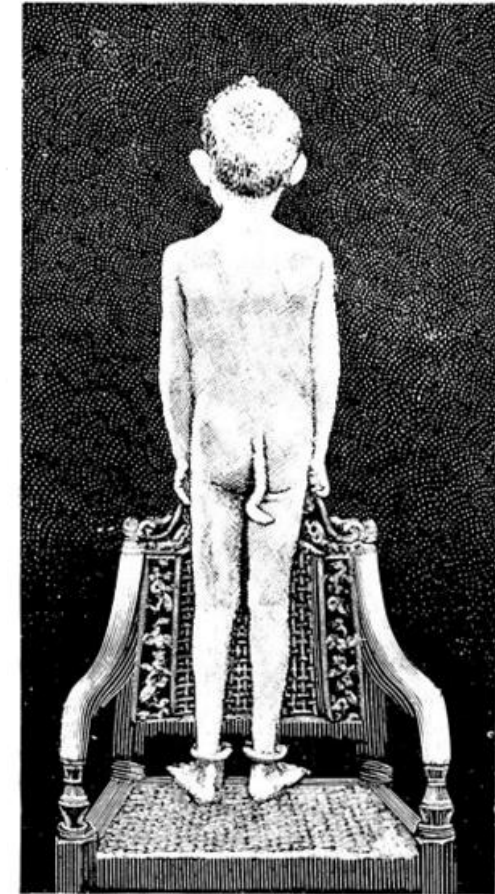
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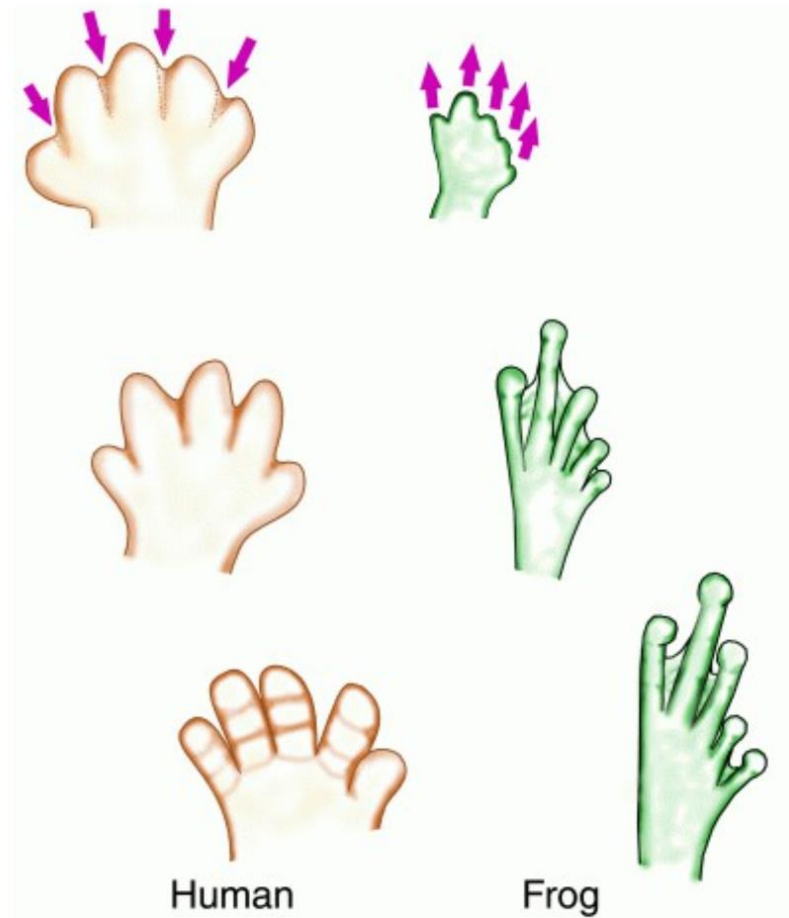
## *Vestigial organs*



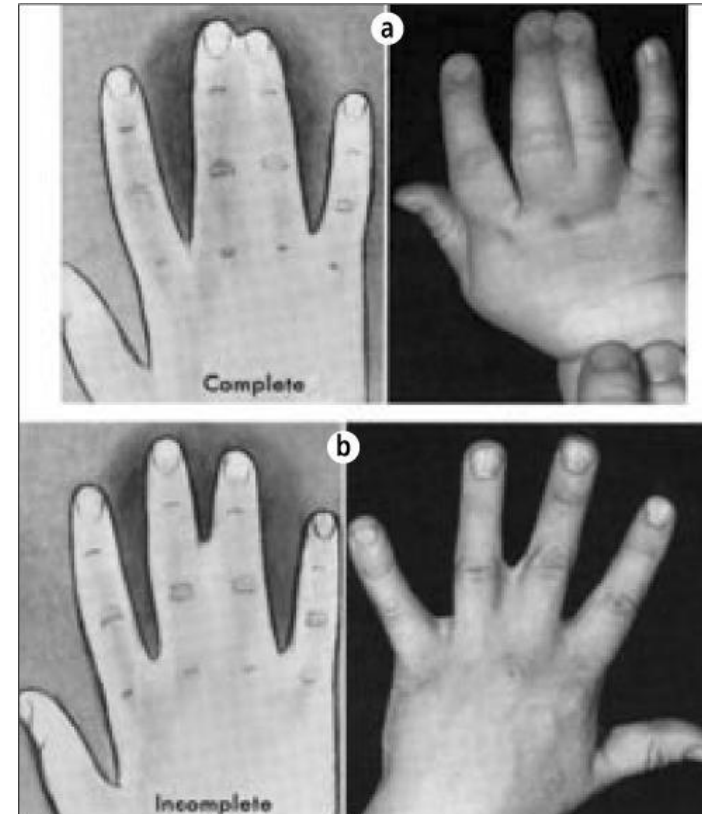


# The other side of the coin: apoptosis

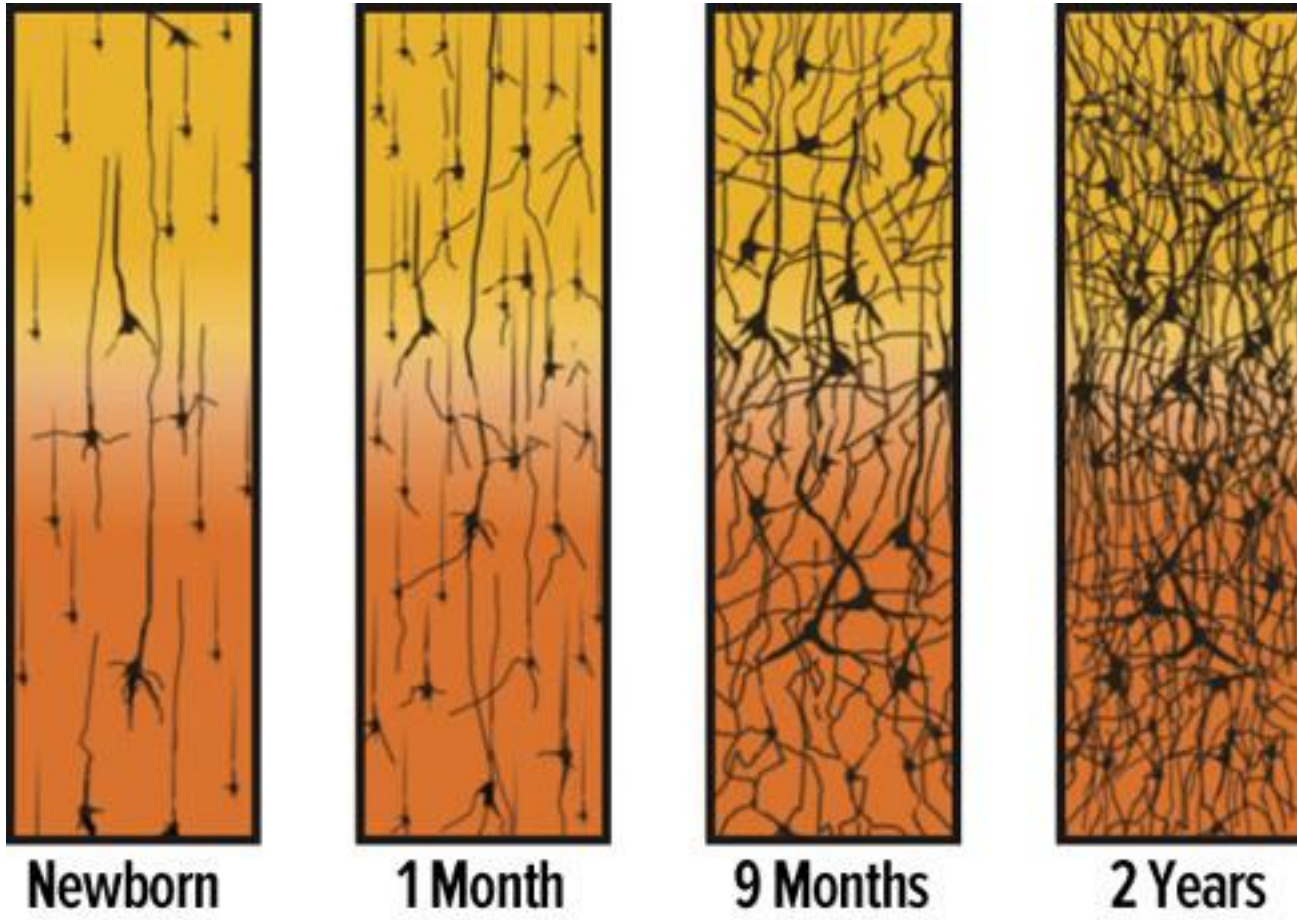
## *Apoptosis vs. out growth*



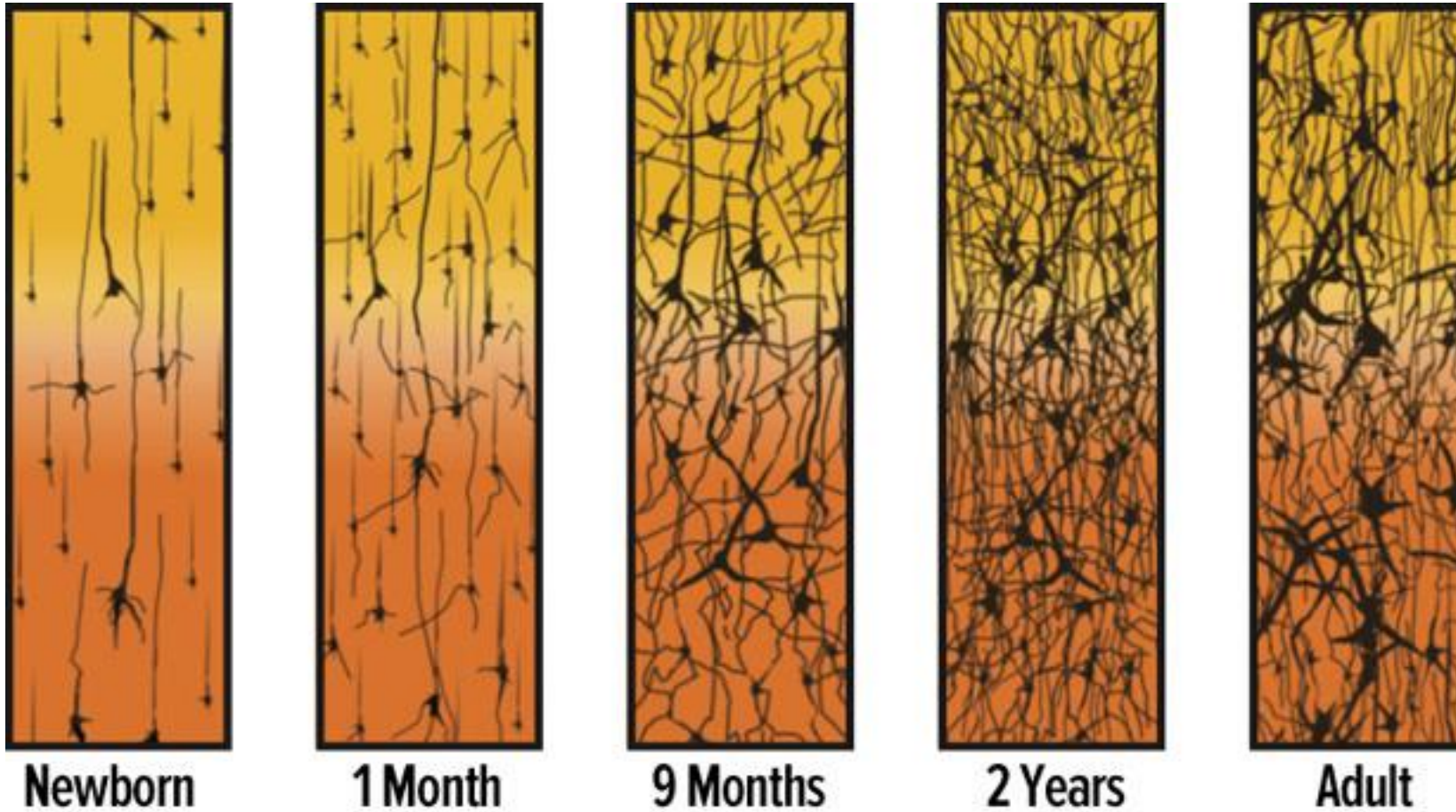
## *Syndactyly* *BMP2 and BMP4*



# The other side of the coin: apoptosis



# The other side of the coin: apoptosis



***Yikes!*** Fewer but stronger synapses

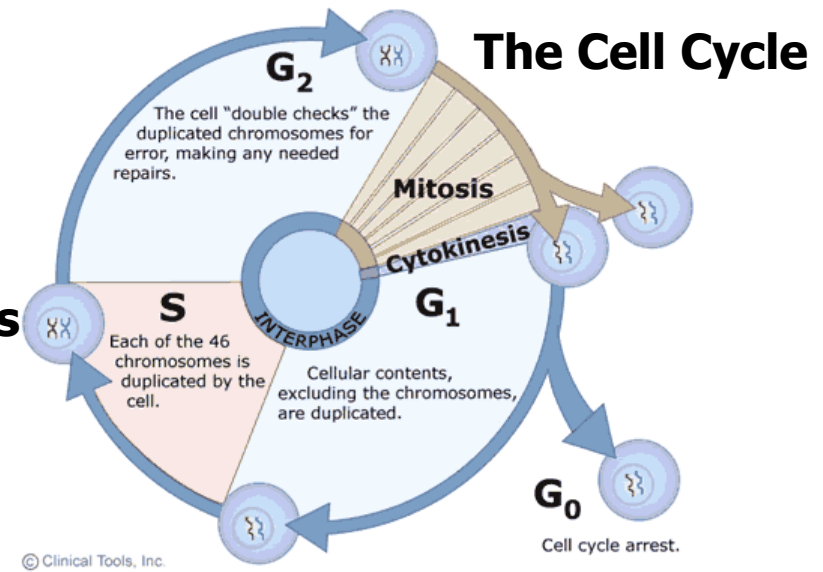
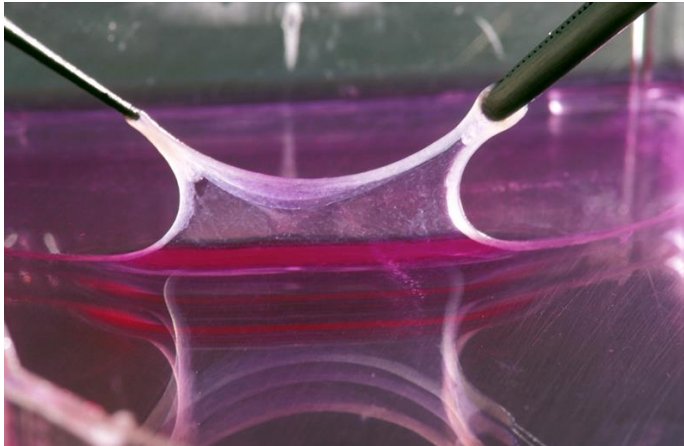


# Rewind and Review

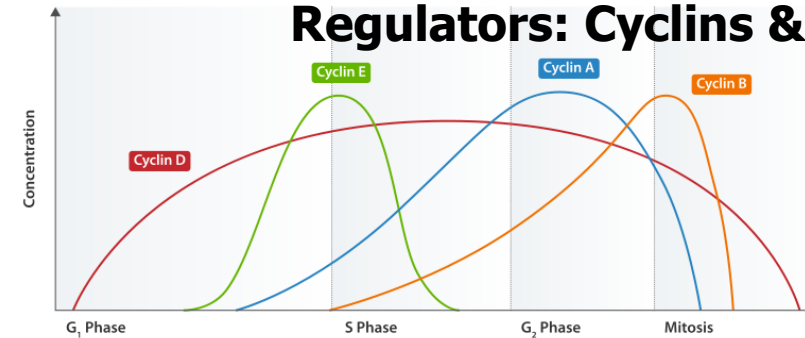
## Order of magnitude for meaningful numbers

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***Typical cellular therapies require  $10^7 - 10^9$  cells***



## Regulators: Cyclins & CDKs



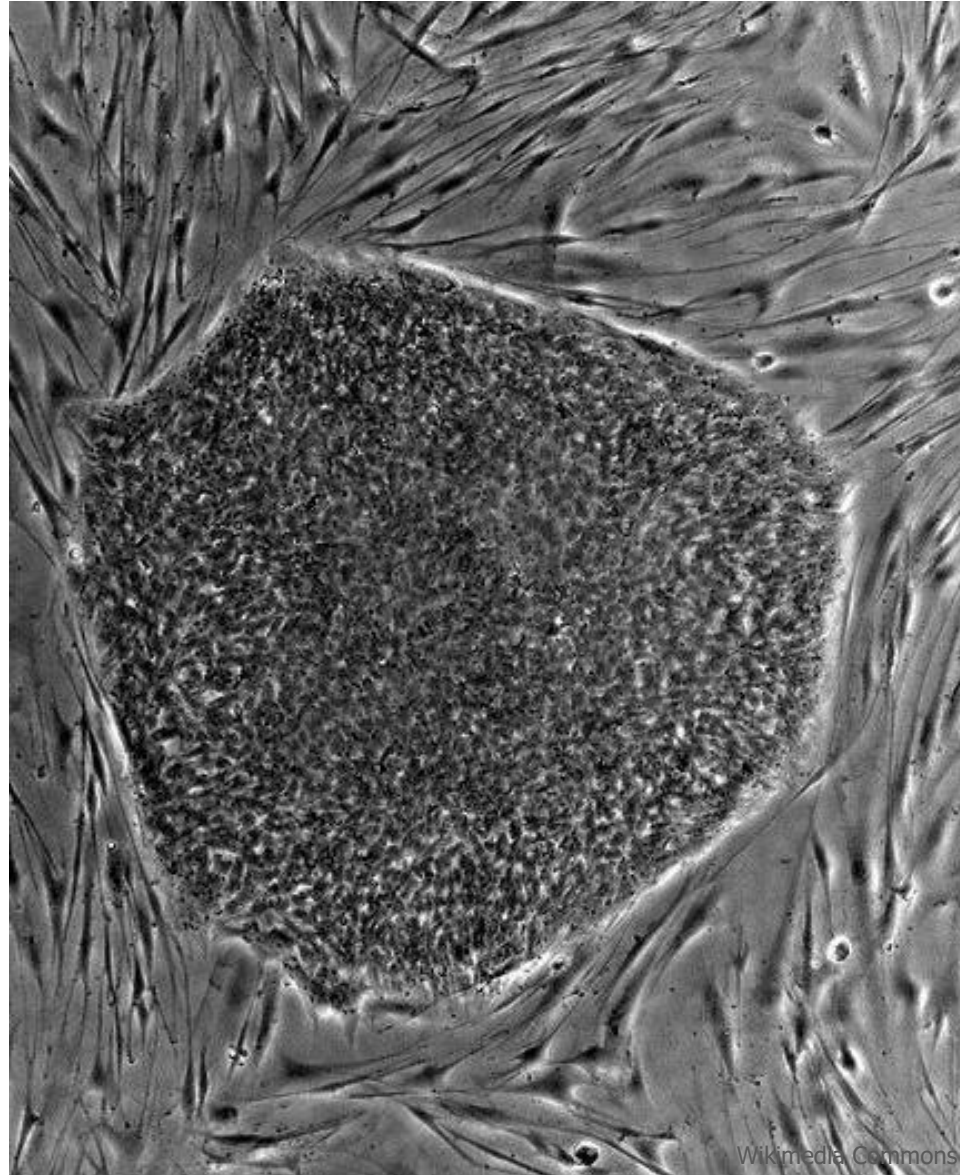
**Development through death**



# Next Lecture

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- Cell Numbers, Growth and Kinetics Part 2: Cell Culture



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