

PERSISTENCE: MATTERS OF LIFE AND DEATH

DKN

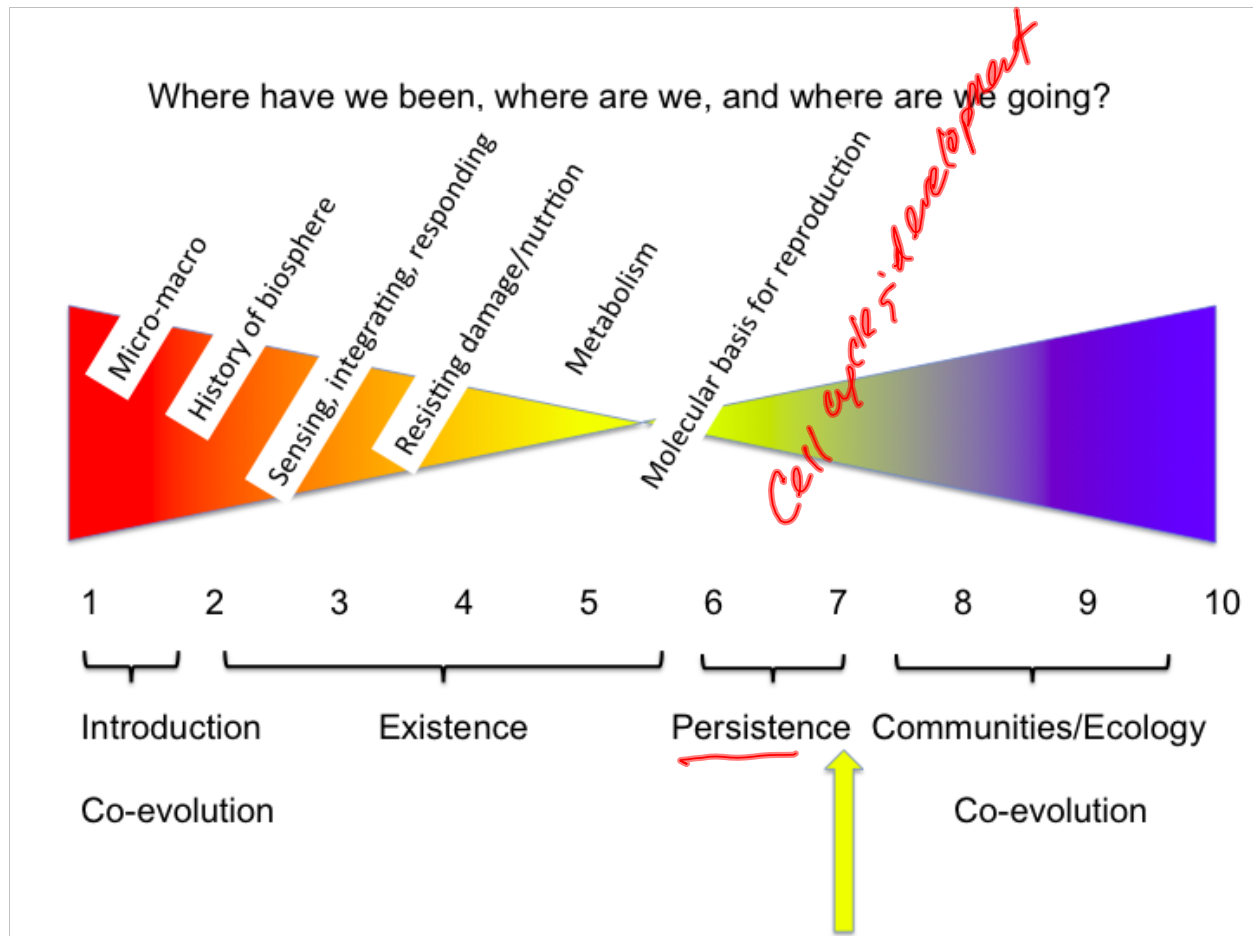
- How do bacteria grow?
- How do they faithfully divide?
- What happens when times get tough?

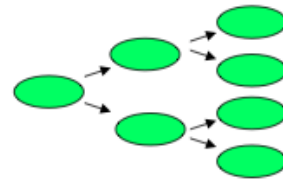
Short break

MMN

- How do you make more cells?
- How do you make germ cells?
- What are the mechanisms of cell death and survival?

EXAM DISCUSSION (15 min)
iClicker Quiz (5min)



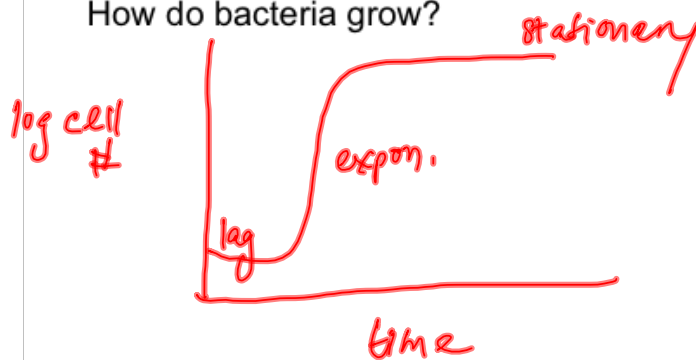


$$y = 2^x$$

→ *E. coli* (20 min gen time)
< 2 days to take over the Earth!

*how do we stop
growth*

How do bacteria grow?



What do individual cells look like?

Rod



E. coli / *B. subtilis*

Coccus



S. aureus

Spirillum



M. magnetum

Vibrio

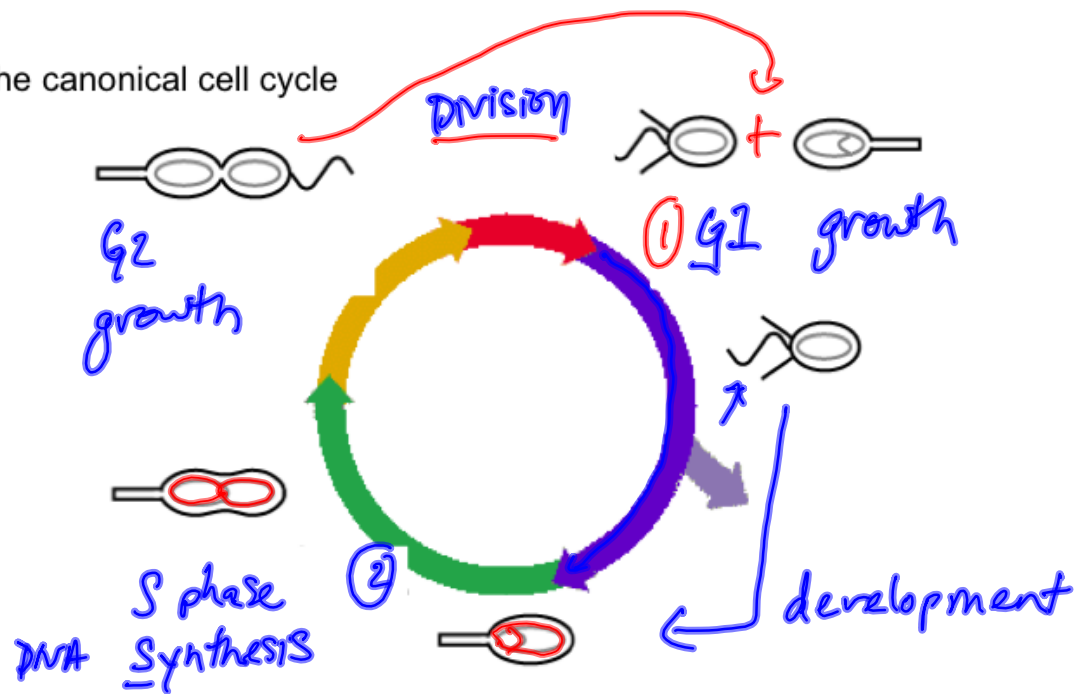


V. harveyi

Example

→ Do they always have the same shape?

The canonical cell cycle

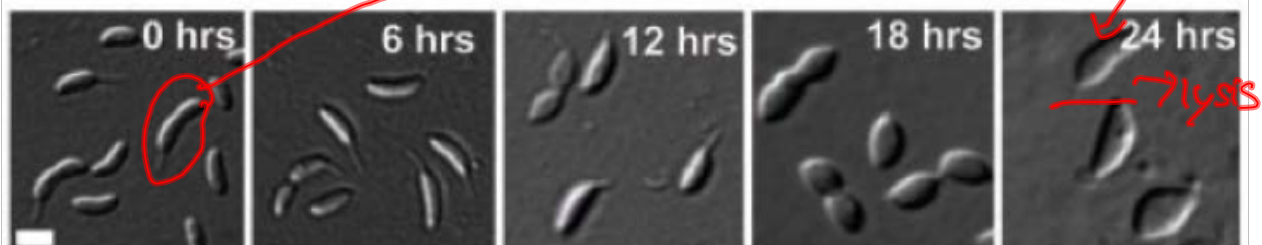


→ A molecular understanding of these processes provides the basis for rational drug design (i.e. new antibiotics)



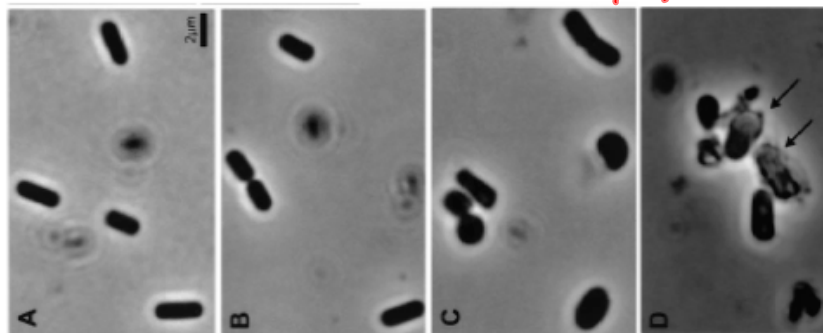
See Prof. Lucy Shapiro iBioSeminar on website, associated papers

Caulobacter, *E. coli* and *B. subtilis* cells depleted of a **key protein** lose their characteristic rod shapes and lyse



C. crescentus

depletion of a key protein →



E. coli or
B. subtilis



depleted →

How do these cells physically grow? (G1)

What structural polymer do all bacteria have?

peptidoglycan

Where does it get inserted as bacteria grow?

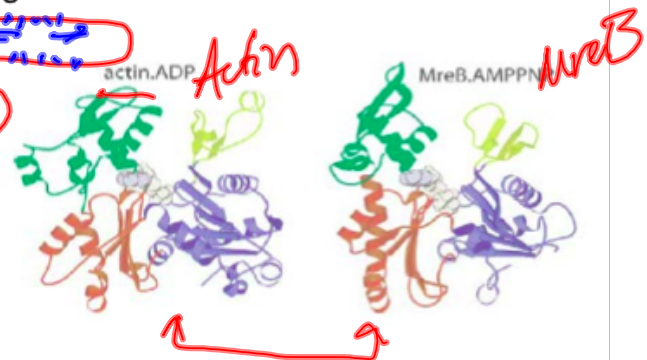
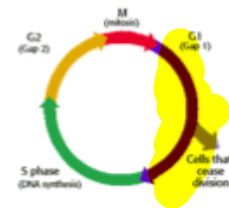
- 1) on edges (elongating)
- 2) at poles (division)

How does it know to go there?

MreB = actin homolog

Where have you seen this actin homolog before?

MamK → magnetosomes in *Chromatium*
holds

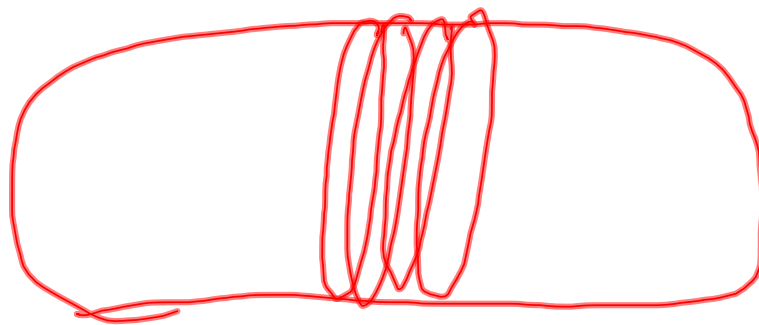


In living cells, MreB-homologs assemble into dynamic helical structures

Let's look at how this works in *B. subtilis*

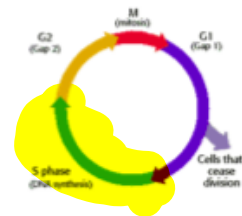
(Animation from D. Rudner and E. Garner, Harvard)

<http://vimeo.com/34377082>

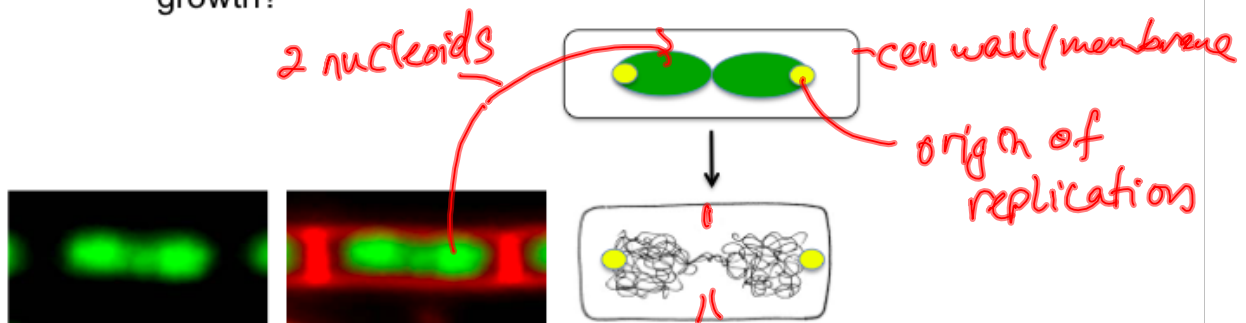


How do cells divide? What about the "S" phase?

→ make copies of everything vital
 ↳ plasmids, chromosome, internal structures



Example: How does *B. subtilis* separate its chromosome during "vegetative" growth?



SpoJ (DNA-binding protein)

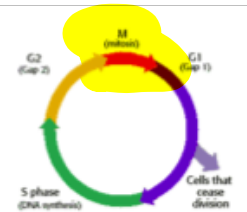
helps direct nucleoids to the poles and segregate chromosome through interactions with other proteins

→ See Prof. Richard Losick's *iBiosseminar* on *B. subtilis* cell division on Bi1 website

What proteins act at the division plane?

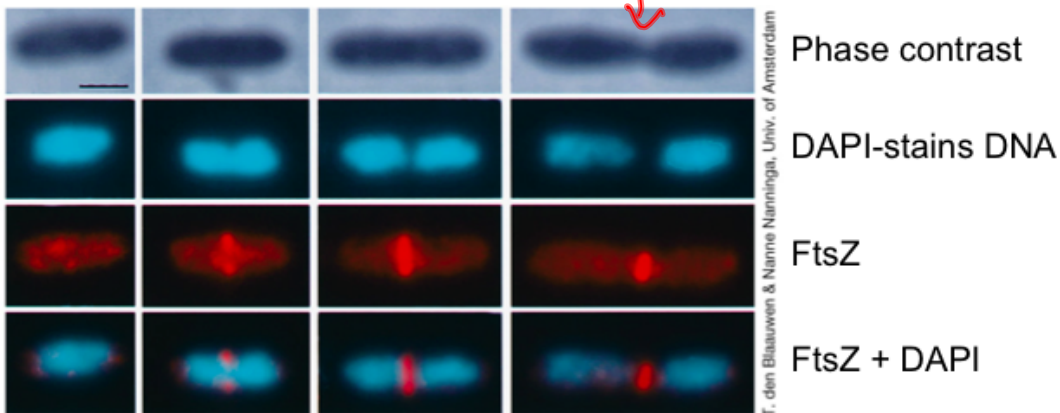
gene

ftsZ – mutant found in fission temperature-sensitive screen



protein FtsZ (tubulin homolog) forms a ring at the site of division

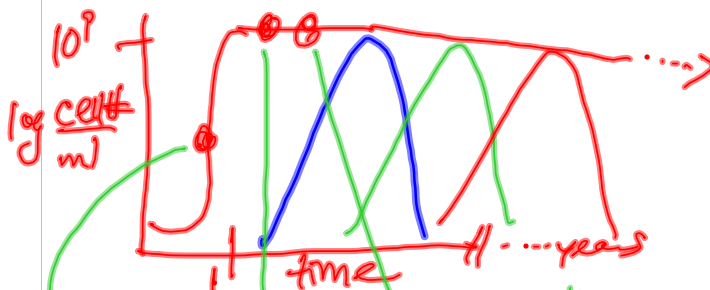
between replicated chromosomes, its constriction drives cell division



growing (G2)

dividing

What happens when times get tough?



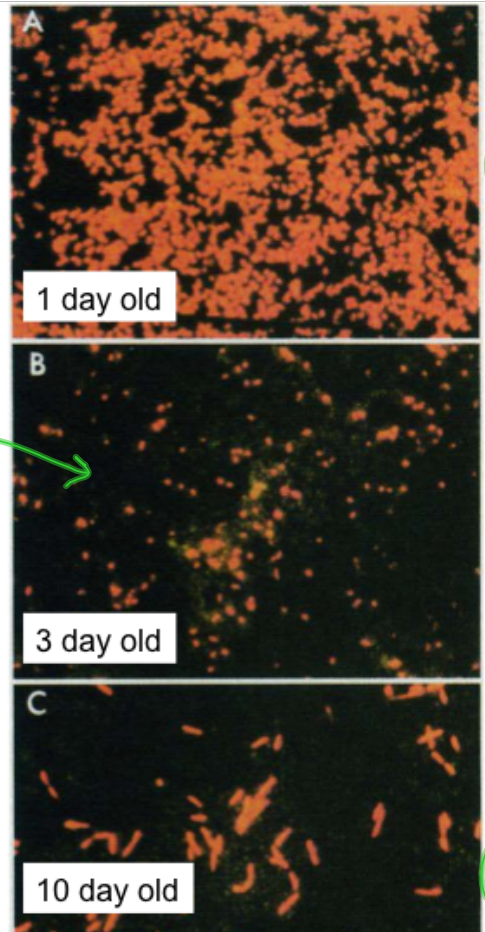
Orange = stains live cells
Green = stains dead cells

What do you notice about the shape?



Growth
Advantage
Stationary
Phase

Zambrano et al, Science 1993



A

C

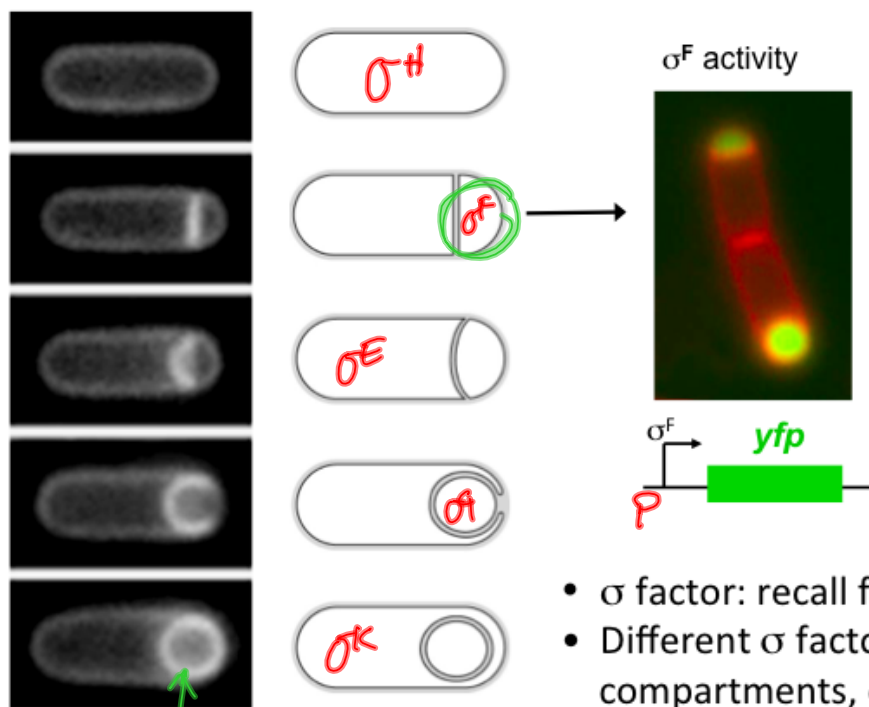
What happens when times get tough?

Let's take a look at *B. subtilis* sporulation in a
time-lapse movie from Michael Elowitz's lab
(Prof. of Biology and Applied Physics, Caltech)



<http://www.elowitz.caltech.edu/publications/GSmovie+phase.avi>

Compartment-Specific Gene Expression During Sporulation



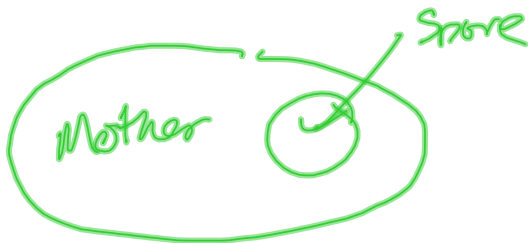
- σ factor: recall from last week
- Different σ factors in different compartments, dynamic!
- Two-way conversation

see spore

Let's take a close-up look using cryoEM
at sporulation in *Acetone**nema longum*

(Grant Jensen's Lab, Caltech):

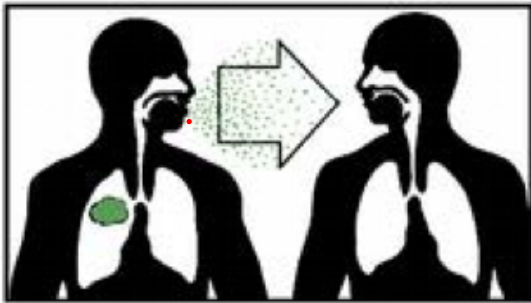
http://lab.jensengroup.org/movies/Acetonema_300MG_final-desktop.mov



What happens when times get tough? *Quiescence*

Mycobacterium tuberculosis (M.tb): 1 in 3 people harbor it worldwide

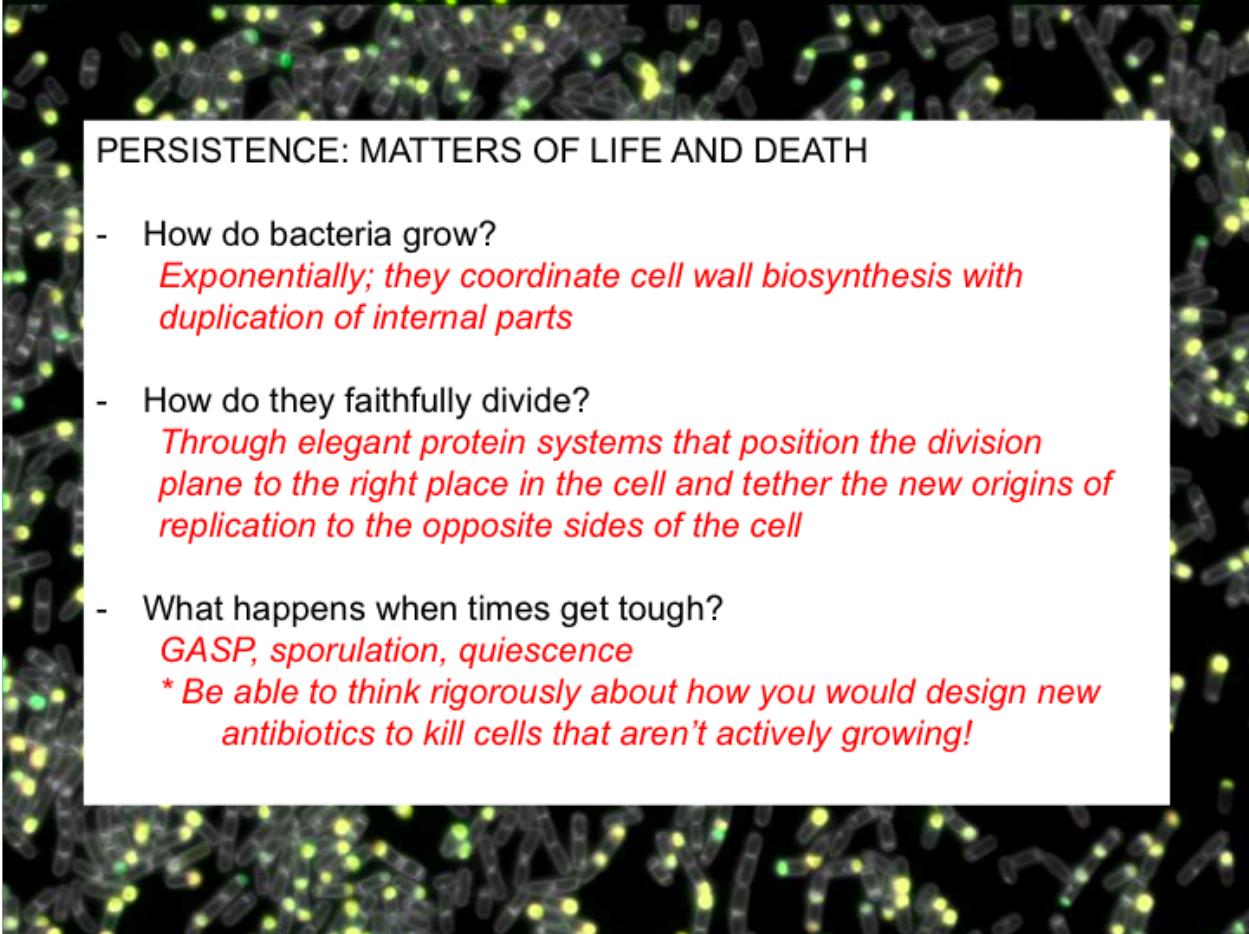
- Spread by coughing (90-95% of cases active disease does not develop → latent state)



- Most antibiotics target growing cells (hit DNA replication, ribosome, peptidoglycan)



- What is the latent state? How could we control it? What experiments might you design to figure this out? ***EXAM Q***



PERSISTENCE: MATTERS OF LIFE AND DEATH

- How do bacteria grow?
Exponentially; they coordinate cell wall biosynthesis with duplication of internal parts
- How do they faithfully divide?
Through elegant protein systems that position the division plane to the right place in the cell and tether the new origins of replication to the opposite sides of the cell
- What happens when times get tough?
GASP, sporulation, quiescence
** Be able to think rigorously about how you would design new antibiotics to kill cells that aren't actively growing!*

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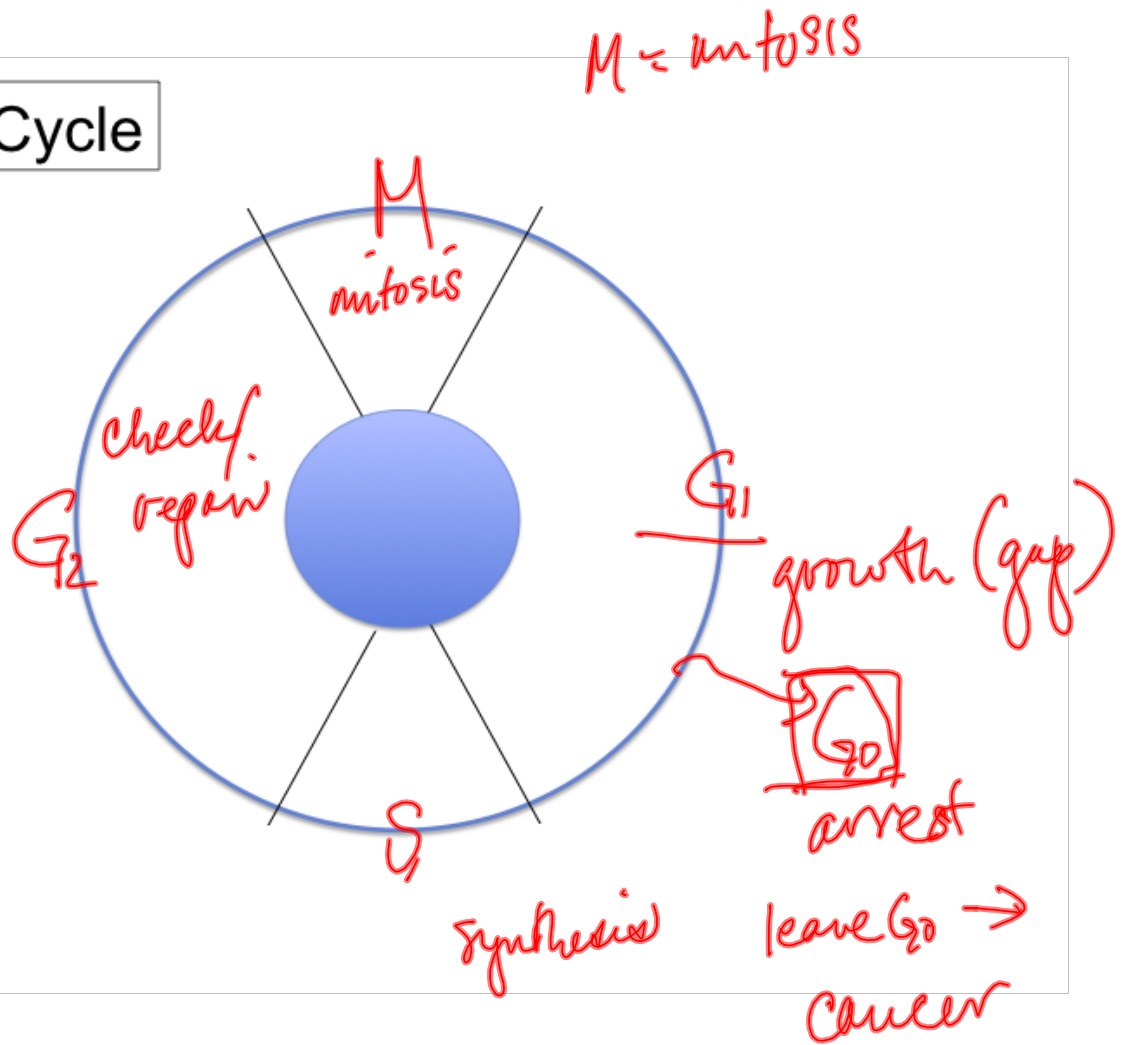
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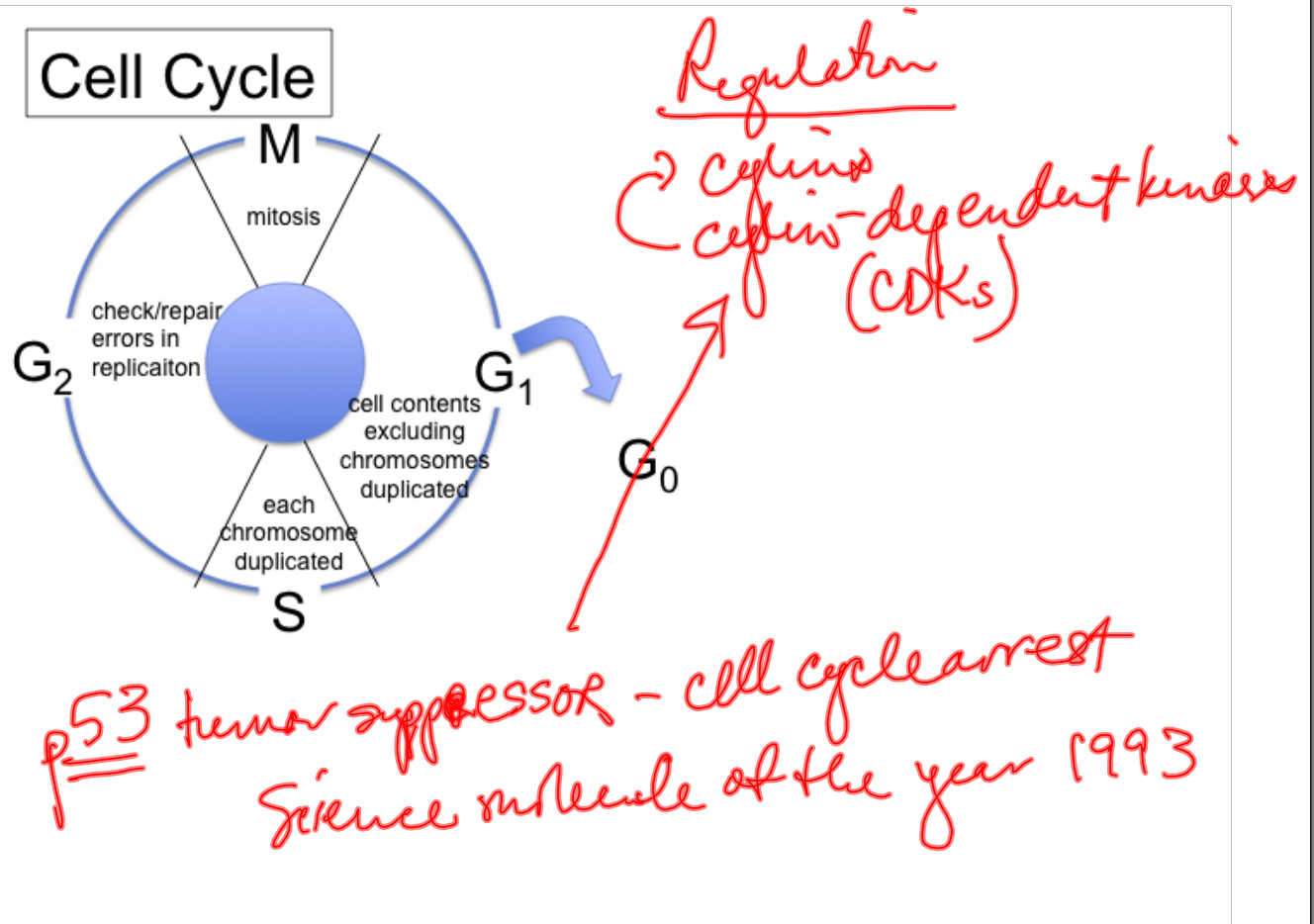


The Lives of a Cell

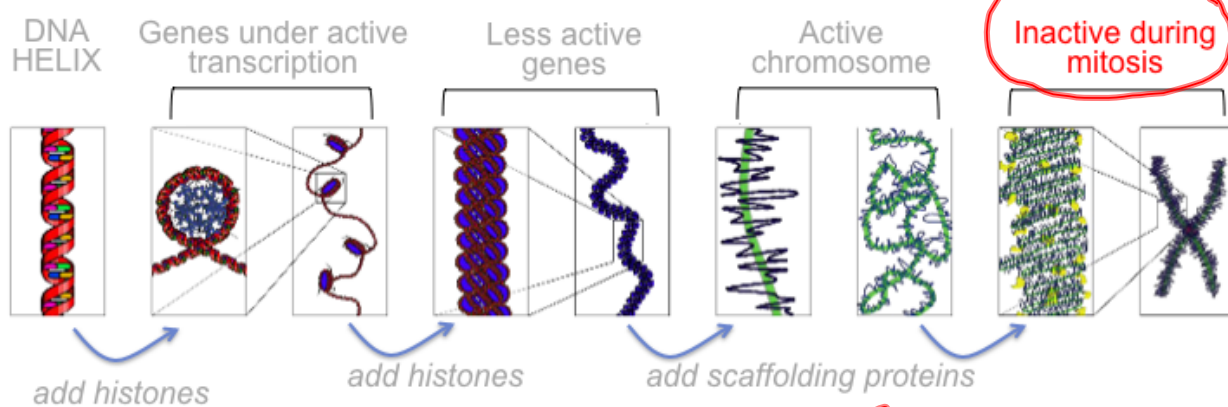
Birth – Growth – Function – Division - Death

Cell Cycle

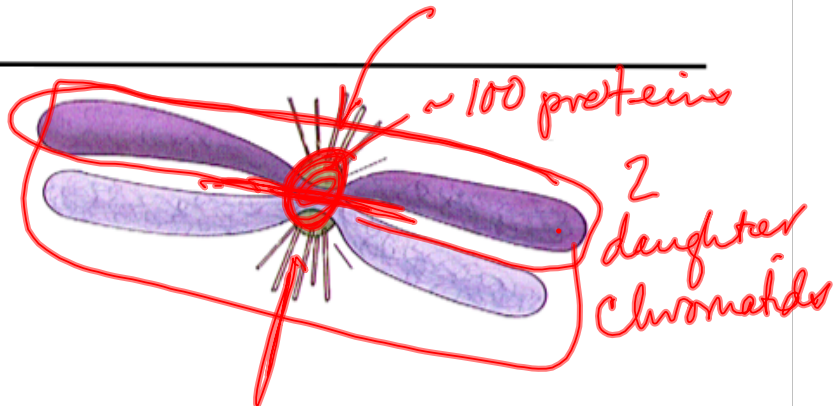




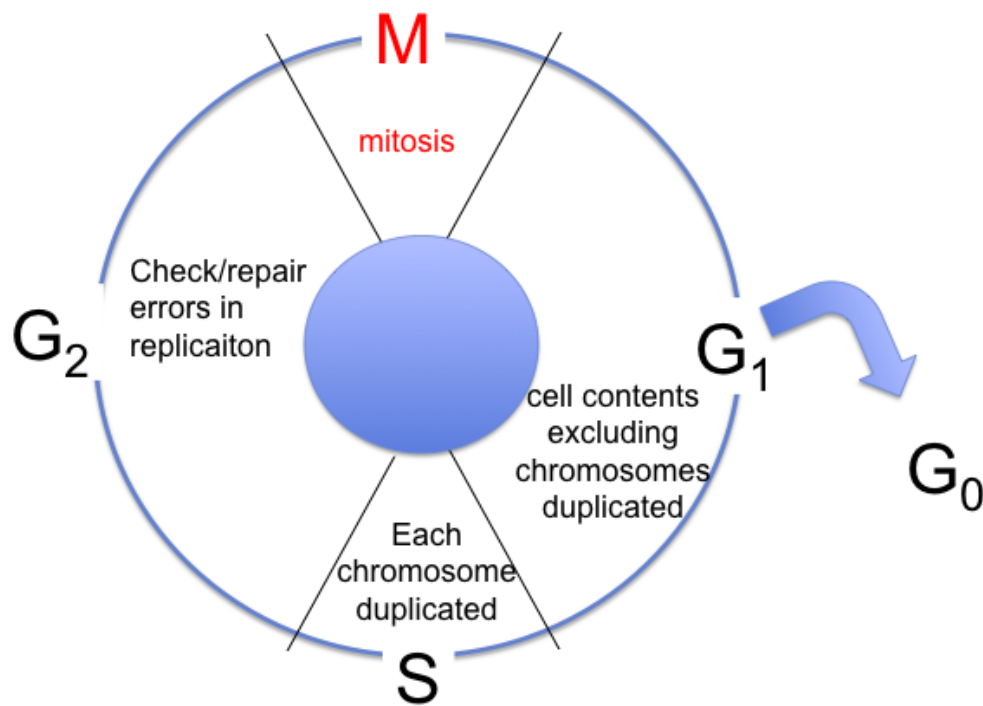
Levels of Organization of the Chromatin/ Chromatin Packing:



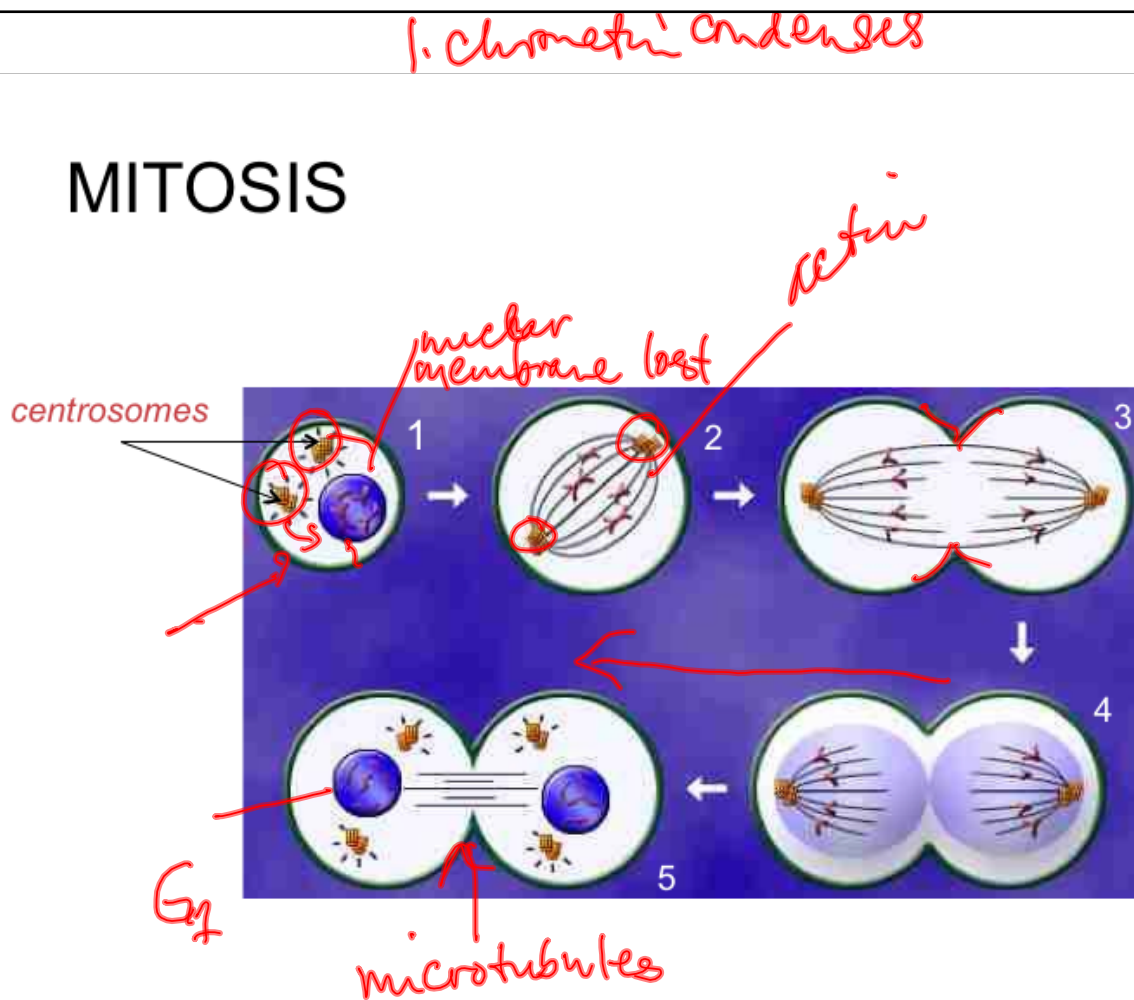
S phase



Mitosis – *cell division, maintaining the same chromosome # ($2n$) diploid*



MITOSIS



Human body
>200 cell types:

Lifespans –

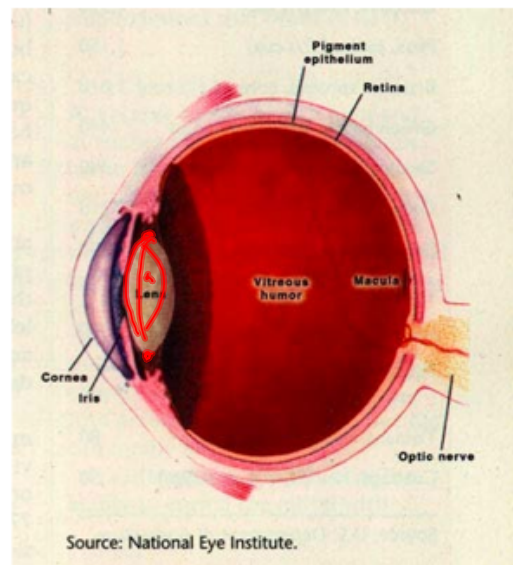
white blood cells

6-7 hrs

red b.c. 120 days

bone cells 25-30 yrs

lens



Plants and Animals have another type of division:

MEIOSIS = 'reduction division' $2n \rightarrow 1n$

In plants often:

gamete production results from mitosis [animals gamete production results from meiosis]

Plant evolution

dominance of $1n$

dominance of $2n$



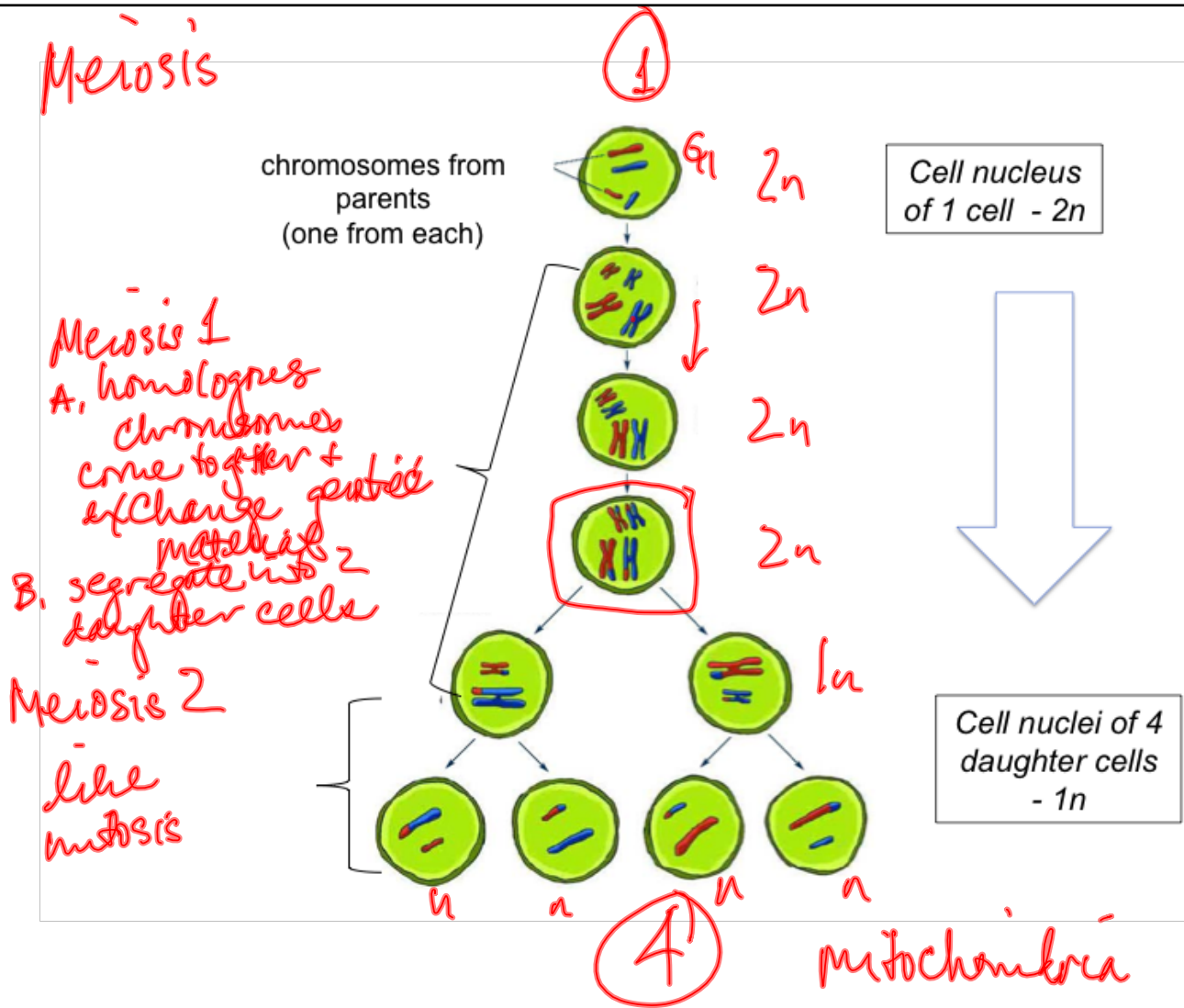
moss



angiosperms

Everything you see is $2n$

Meiosis





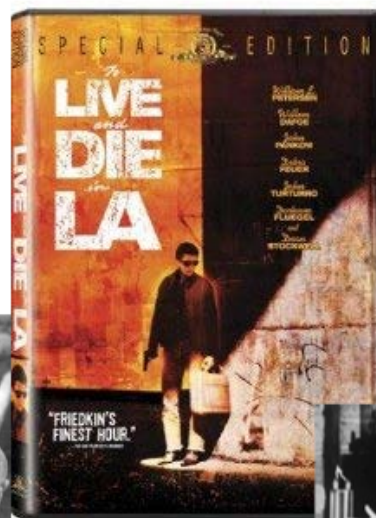
Barbara McClintock
(1902-1992)

Discovered crossing over
during meiosis 1

exchange
of
genetic
material

Nobel Prize in Physiology/Medicine 1983

To Live or Die? Apoptosis/Autophagy

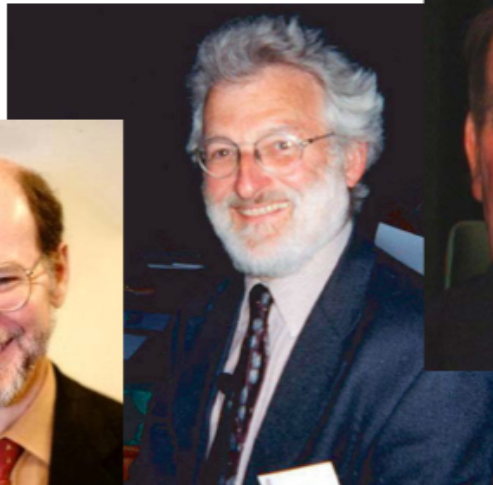


Apoptosis – Programmed Cell Death

Nobel Prize in Medicine 2002



Robert Horvitz - MIT



Sir John Sulston
Wellcome Trust/Sanger Inst



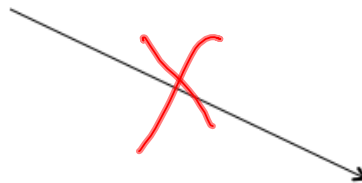
Sydney Brenner
UC Berkeley

PubMed 1972- 1979 – 36
2012 alone > 11,000

Apoptosis

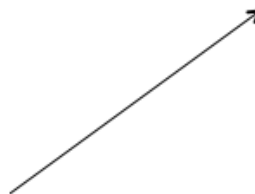
Tightly controlled death

[Important in responses to stress
and during development/
maintenance of tissues]



Necrosis

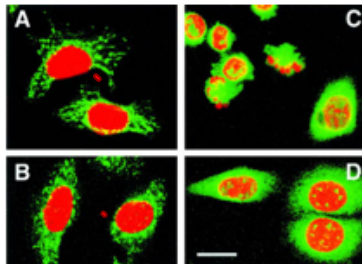
Uncontrolled death & destruction



INFLAMMATION

Characteristics of Apoptosis

- Early - Mitochondria targeted - Increase in mitochondrial membrane permeability



Uninduced

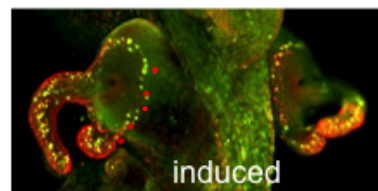
Induced

Cytochrome c of mitochondria released into the cytoplasm [nuclei]

- Cells shrivel/round
chromatin condenses/DNA fragments

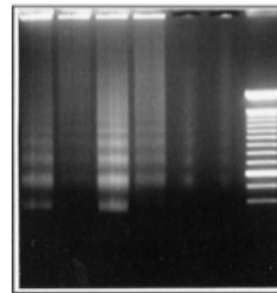


uninduced



induced

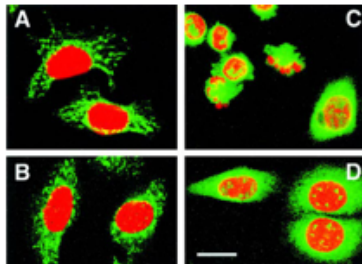
Condensed chromatin



DNA fragmentation into
~180 bp bits

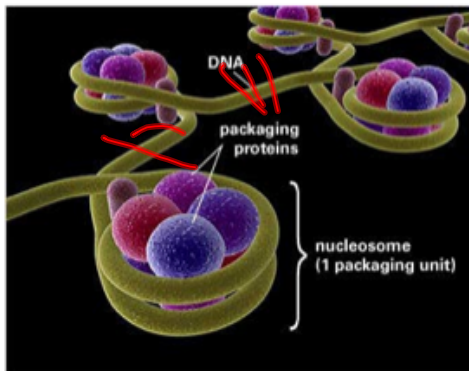
Steps in Apoptosis

- Mitochondria targeted - Increase in mitochondrial membrane permeability

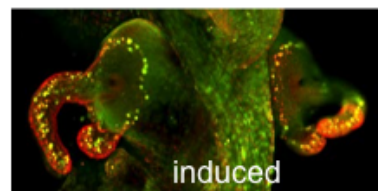
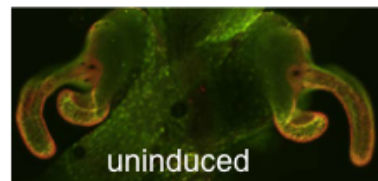


Uninduced Induced

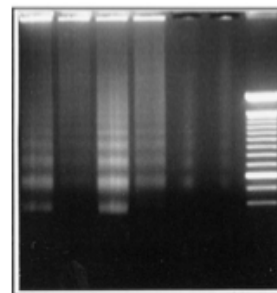
Cytochrome c of mitochondria released into the cytoplasm [nuclei]



-Cells shrivel/round
chromatin condenses/DNA fragments

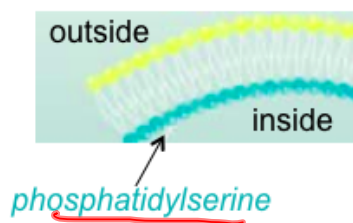


Condensed chromatin



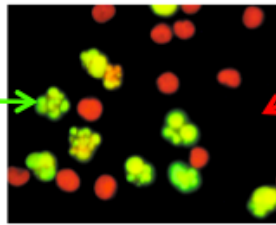
DNA fragmentation into
~180 bp bits

Surface/shape changes in apoptosis



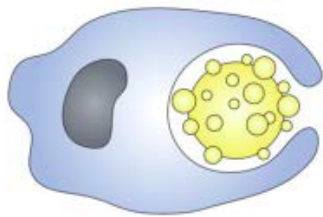
Membrane polarity lost

apoptotic cell



Living cell

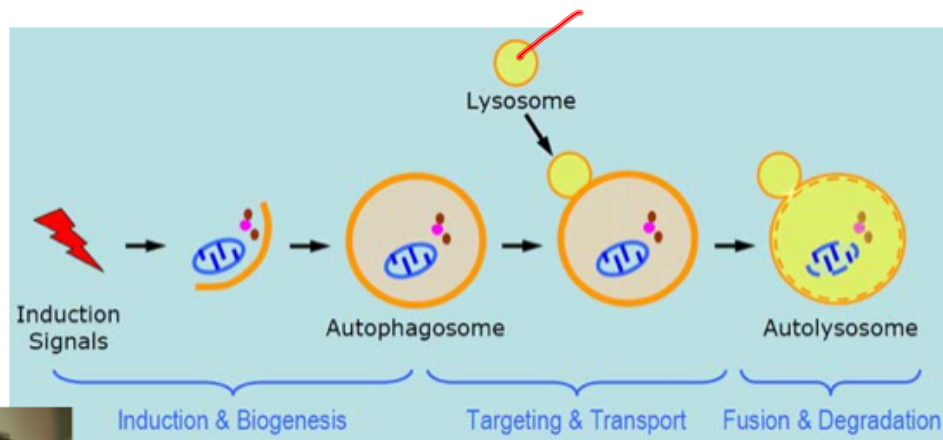
Blebbing



Phagocytosis of the corpse

Alternative pathway -

Autophagy: degradation of a cell's components by its own machinery



Jennifer Lippincott-Schwartz
National Institutes of Health

The membranes of autophagosome
come from mitochondrial
membranes !!!

PERSISTENCE: MATTERS OF LIFE AND DEATH

MMN

- How do you make more cells?
THROUGH MITOSIS
- How do you make germ cells?
THROUGH MEIOSIS
- What are the mechanisms of cell death and survival?
Two options are programmed cell death and survival
through autophagic processes



**EVERYTHING
IS GOING TO BE
ALRIGHT**
MAYBE NOT
TODAY BUT
EVENTUALLY

Questionnaire



- Only 6 people have responded (thank you to those who did so constructively)
- You have until Wednesday lunchtime to return your surveys to your house ombudsperson or Molly.

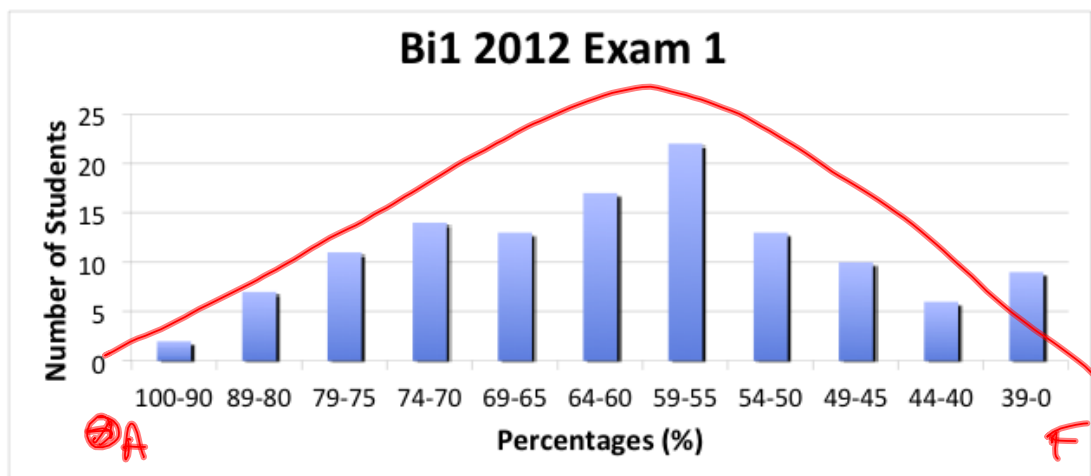
Exam

What were the goals?

Provide you with an incentive to learn how to:

- 1.) integrate information
- 2.) critically read and write about scientific research
- 3.) manage your time in a scholarly fashion

Class midterm performance:



- Exams will be handed back in section or you can contact your section TAs to pick up your exam earlier.
- Keys will be posted on the class website.
- If you want to talk to us about the exam, please make an appointment.

↓ Is this disappointing? **Yes** Grim? **No** Here's the deal on overall midterm grades and beyond:

15%: Writing Assignment

ONLY the hypothesis was counted for this. Once the rough draft and final drafts are graded, this part of your grade will change significantly.

20%: iClicker Quizzes

This is before we drop one quiz score but WITH the two extra credit assignments. You still have a shot to improve by taking advantage of extra credit opportunities and consistently reviewing past material.

15%: Homework

This is BEFORE we drop your lowest homework score and counts only Homeworks 1-4. Realize that you still have 4 homework assignments to add to this category.

30%: Exam 1+2

↑ To give you a chance for redemption, your final grade in this category will be calculated as the better of (15% Exam 1 + 15% Exam 2) OR (10% Exam 1 + 20% Exam 2).

20%: Final

↑ We gave everyone 100% in this category because most of you are on track to opting out of the final. Good job!

Midterm Grades:

A: 100-93%

A-: 92.9-90%

B+: 89.9-87%

B: 86.9-83%

B-: 82.9-80%

C+: 79.9-77%

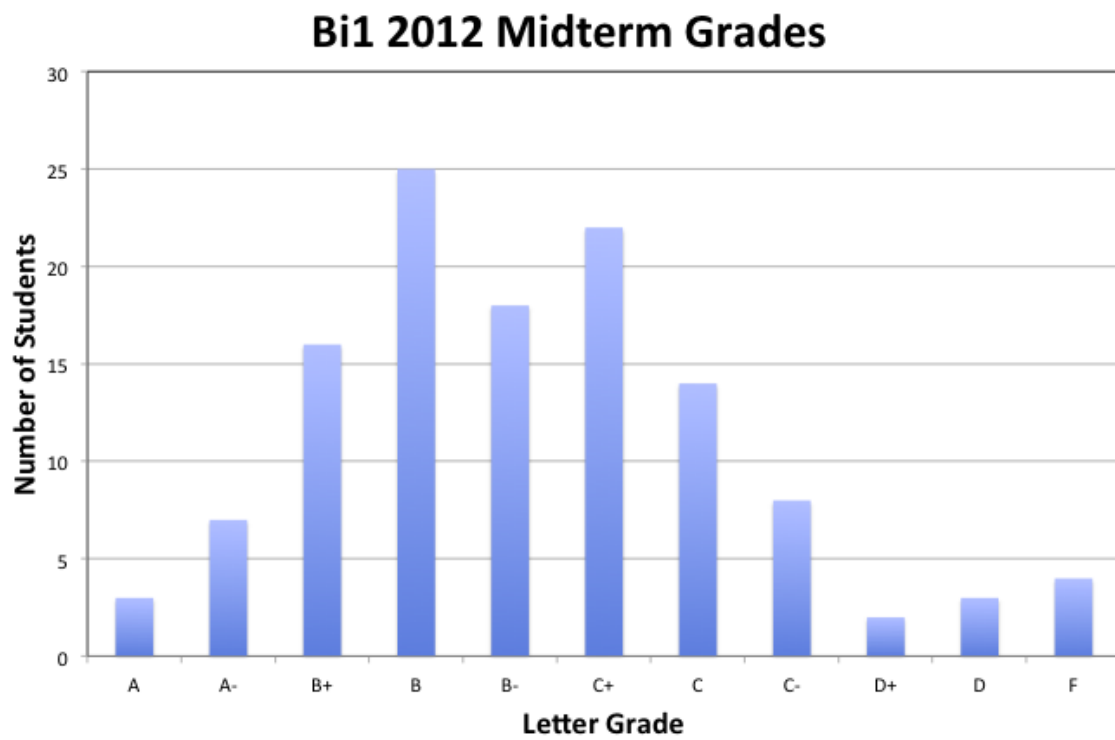
C: 76.9-73%

C-: 72.9-70%

D+: 69.9-67%

D: 66.9-63%

F: 62.9-1%



TAKE HOME MESSAGES

- **Use your resources:** Prof. Newman, Prof. McFall-Ngai, Dr. Sullivan, all 23 (!!) TAs with office hours every day of the week *and* by appointment. You have no excuse if you don't seek help. *Not seeking help is one of the most common mistakes frosh make and later regret. Don't fall into that trap.*
- **We can help you improve:**
 - * Your ability to integrate the information
 - * Your ability to critically read a paper
 - * Your strategies for time management (*we've been there, and it only gets more challenging the older you get...*)

This class emphasizes integration, critical thinking and writing. These are arguably more important skills for your future success (regardless of your major) as your ability to crank through problem sets in your other classes. Don't blow this class off because it is different.