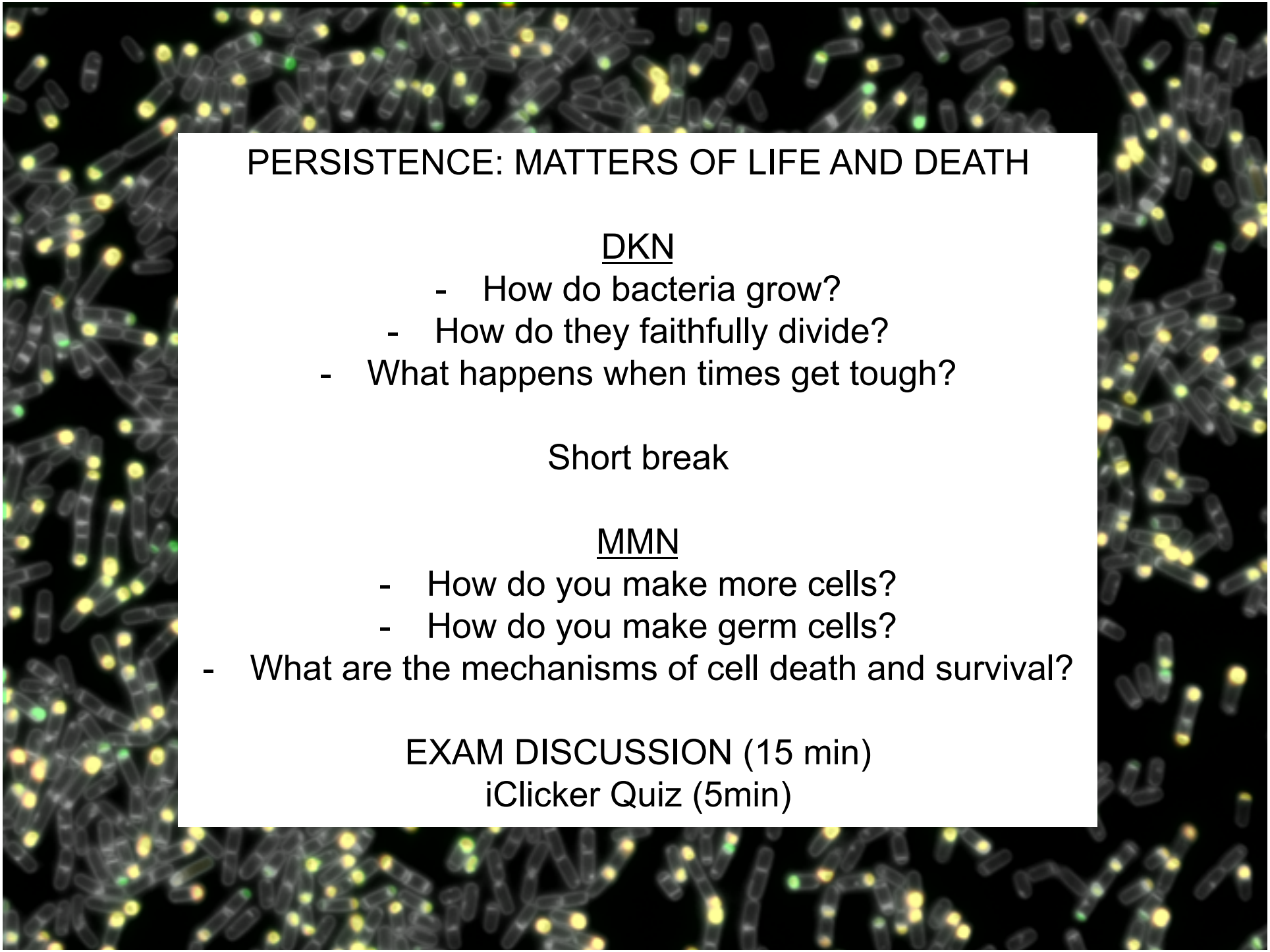


PERSISTENCE: MATTERS OF LIFE AND DEATH

DKN

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

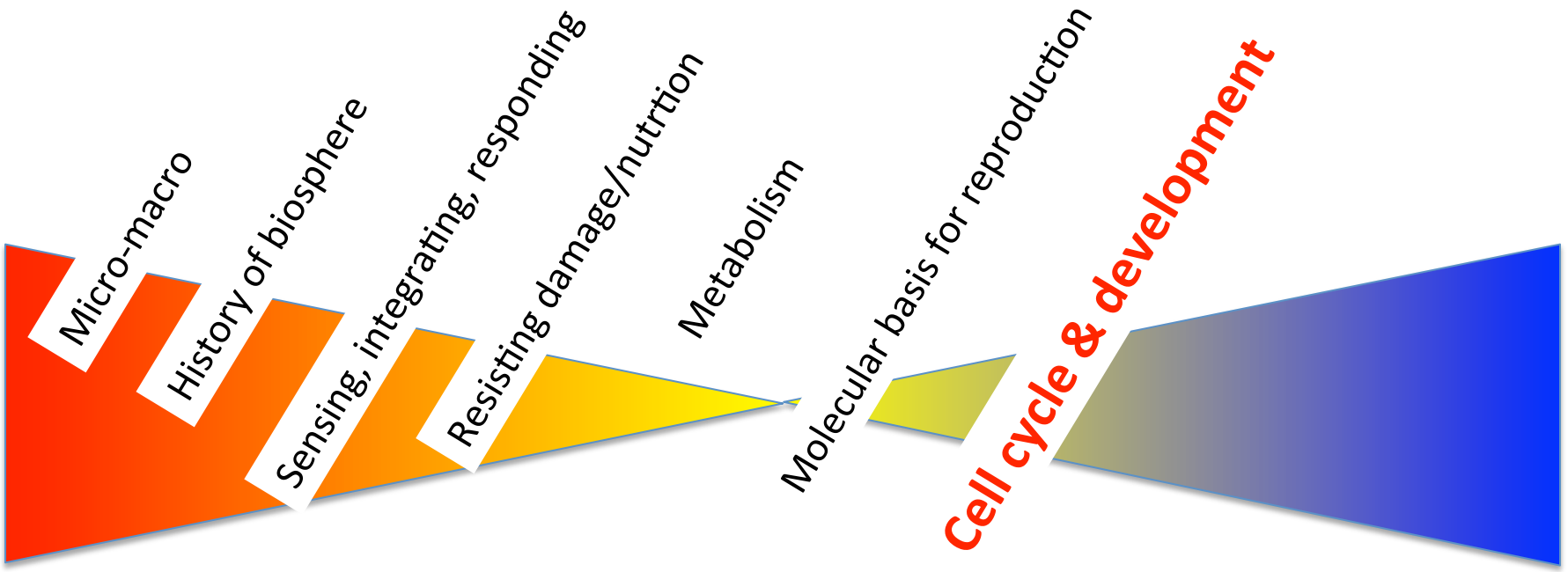
Short break

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)

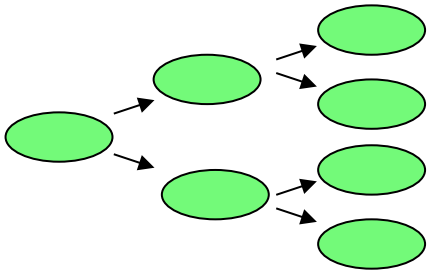
Where have we been, where are we, and where are we going?



1 2 3 4 5 6 7 8 9 10

Introduction Existence Persistence Communities/Ecology

Co-evolution Co-evolution



$$y = 2^x$$

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

How do we stop them?

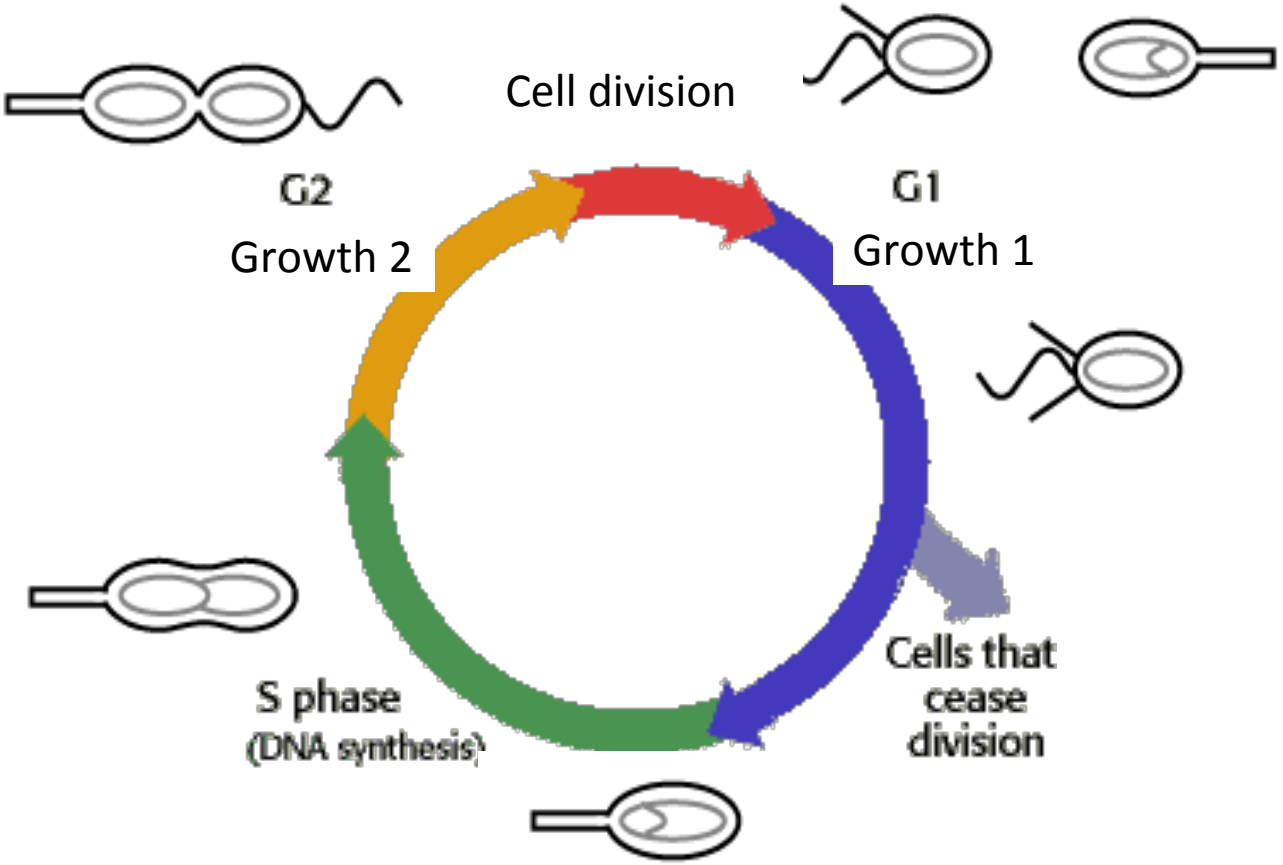
How do bacteria grow?

Draw growth curve

What do individual cells look like?

DRAW BASIC SHAPES – rods, vibrios, cocci, spirilla, make a note that Cell shape changes with age!! (will return to at the end…)

The canonical cell cycle

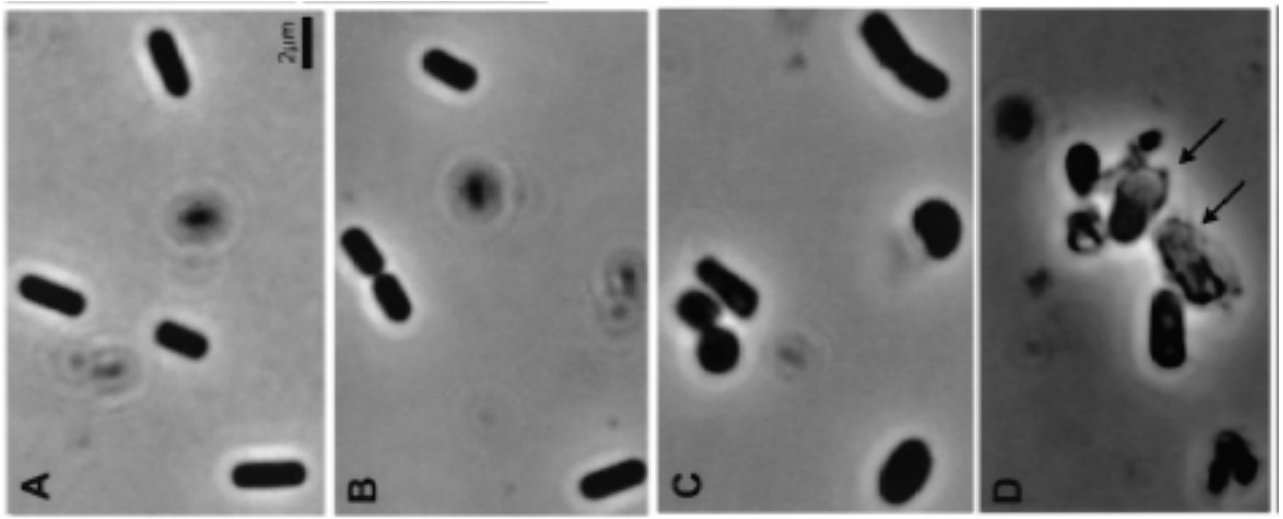


→ A molecular understanding of these processes provides the basis for rational drug design (*i.e.* new antibiotics)

See Prof. Lucy Shapiro's iBioSeminar on BI1 website, associated papers

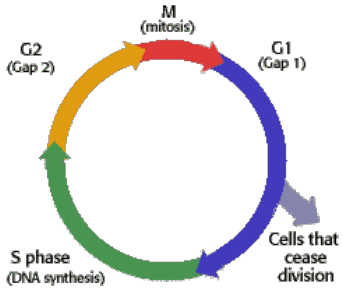
Caulobacter, *E. coli* and *B. subtilis* cells depleted of a key protein lose their characteristic rod shapes and lyse

C. crescentus



E. coli *B. subtilis*

How do these cells physically grow? (G1)



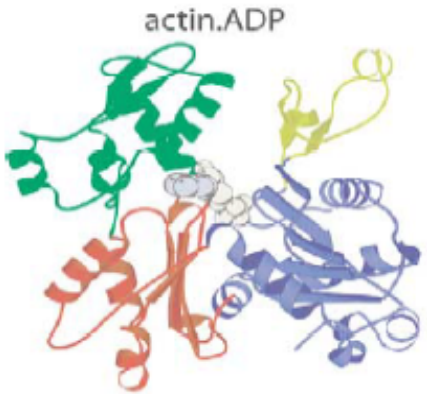
What structural polymer do all bacteria have (cell wall)?

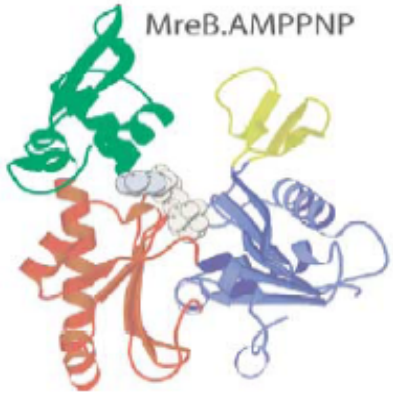
PEPTIDOGYLAN

Where does it get inserted as bacteria grow?

- On edges (elongation) then at poles (during division)

How does it know to go there?





MreB protein (an actin homolog)
positions peptidoglycan synthases

Where have you seen this actin homolog before?

Magnetosome paper!

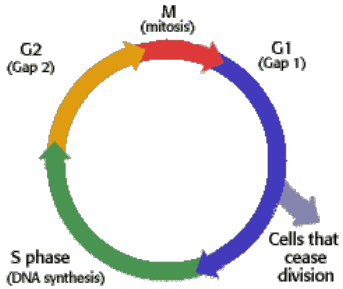
In living cells, MreB-homologs assemble into dynamic helical structures

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?

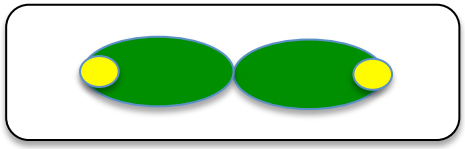


Have to make copies of everything

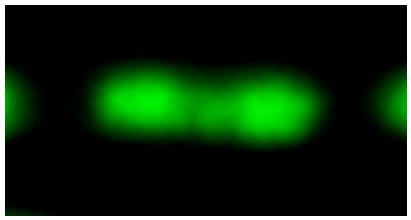
Plasmids, chromosome, internal structures (*magnetosomes,* etc…)

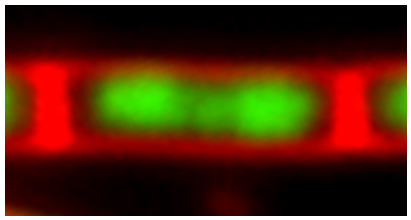
Example: How does *B. subtilis* separate its chromosome during vegetative growth?

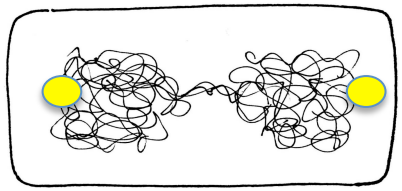
nucleoids



Origin of replication



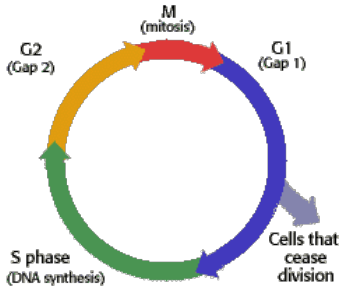




Spo0J (DNA-binding protein) helps anchor nucleoids to the poles, anaolgous to a centromere, later pulled by actin-like filaments to segretate chromosome

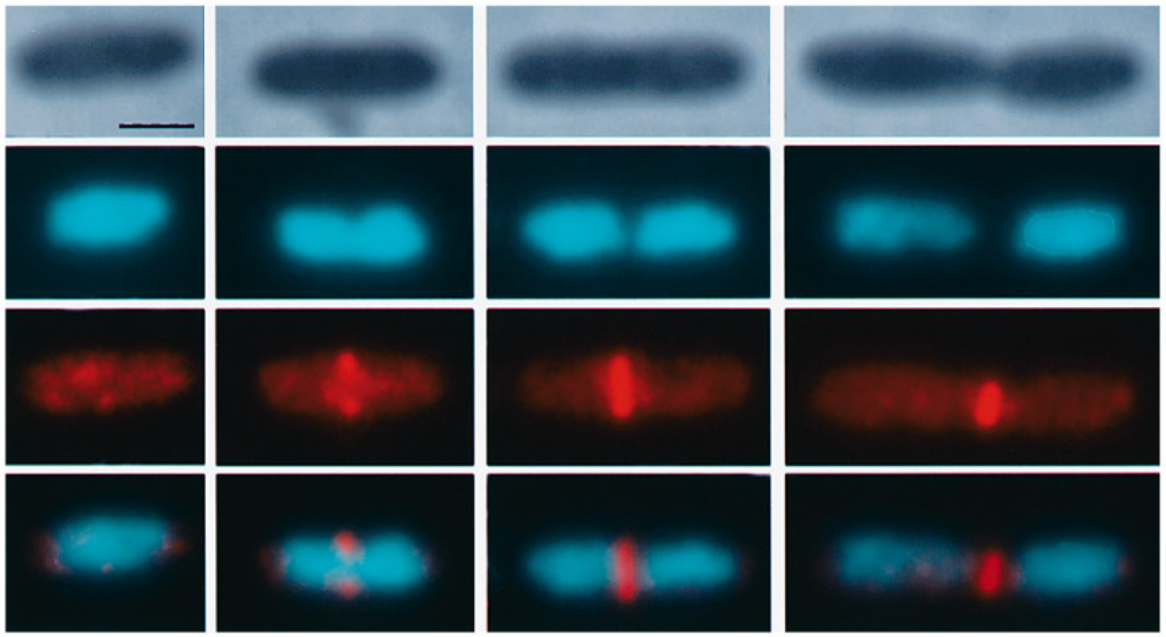
→ See Prof. Richard Losick’s *iBioseminar* on *B. subtilis* cell division on Bi1 website

What proteins act at the division plane?



ftsZ – mutant found in fission temperature-sensitive screen

FtsZ (tubulin homolog) forms a ring at the site of division between replicated chromosomes, its constriction drives cell division



Phase contrast

DAPI-stains DNA

FtsZ

FtsZ + DAPI

(b)

division

What happens when times get tough?

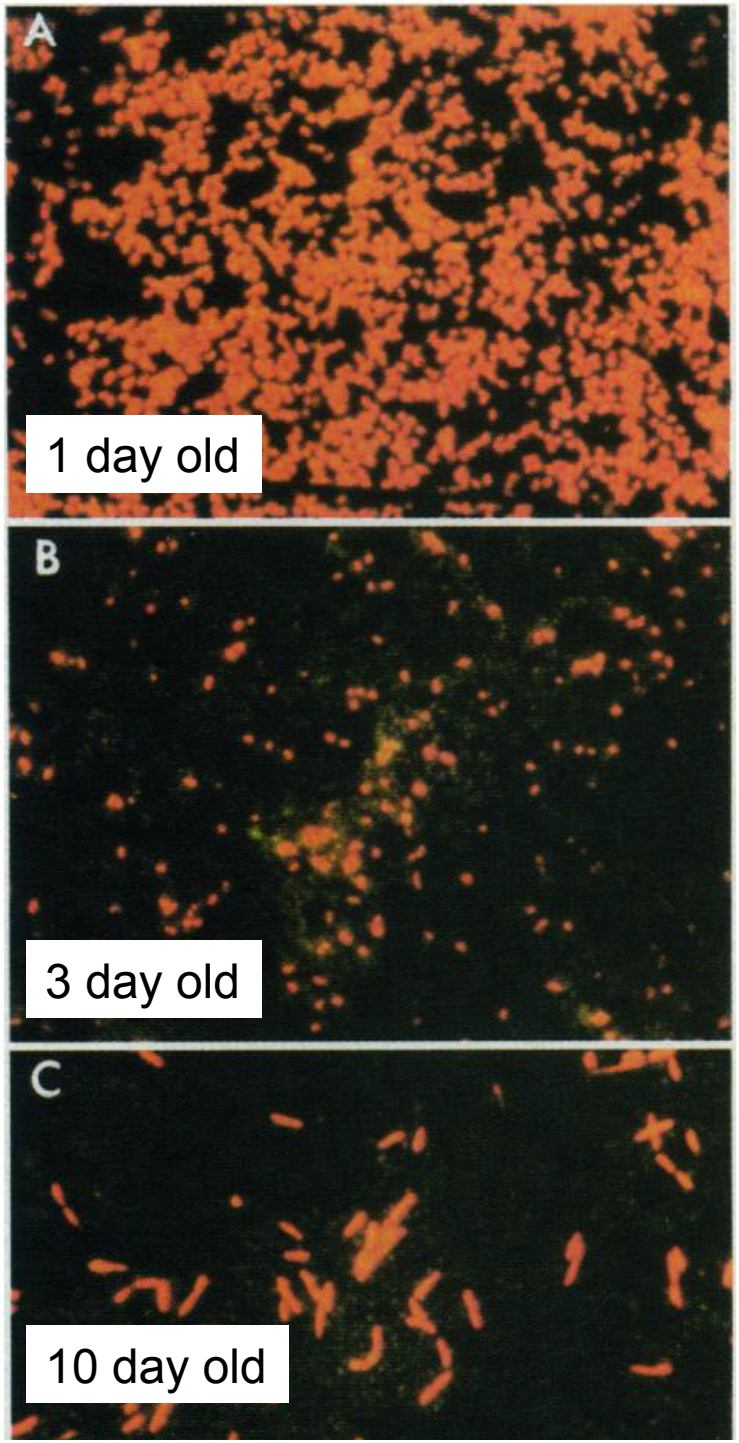
Draw classical GASP curve, with waves of takeovers – map to cell shape below

Orange = stains live cells
Green = stains dead cells

What do you notice about the shape?
E.coli goes from rods to cocci (and back again with GASP)

Growth
Advantage
Stationary
Phase

Zambrano et al, Science 1993



What happens when times get tough? Sporulation

Let's take a look at sporulation in a time-lapse Movie from Michael Elowitz's lab

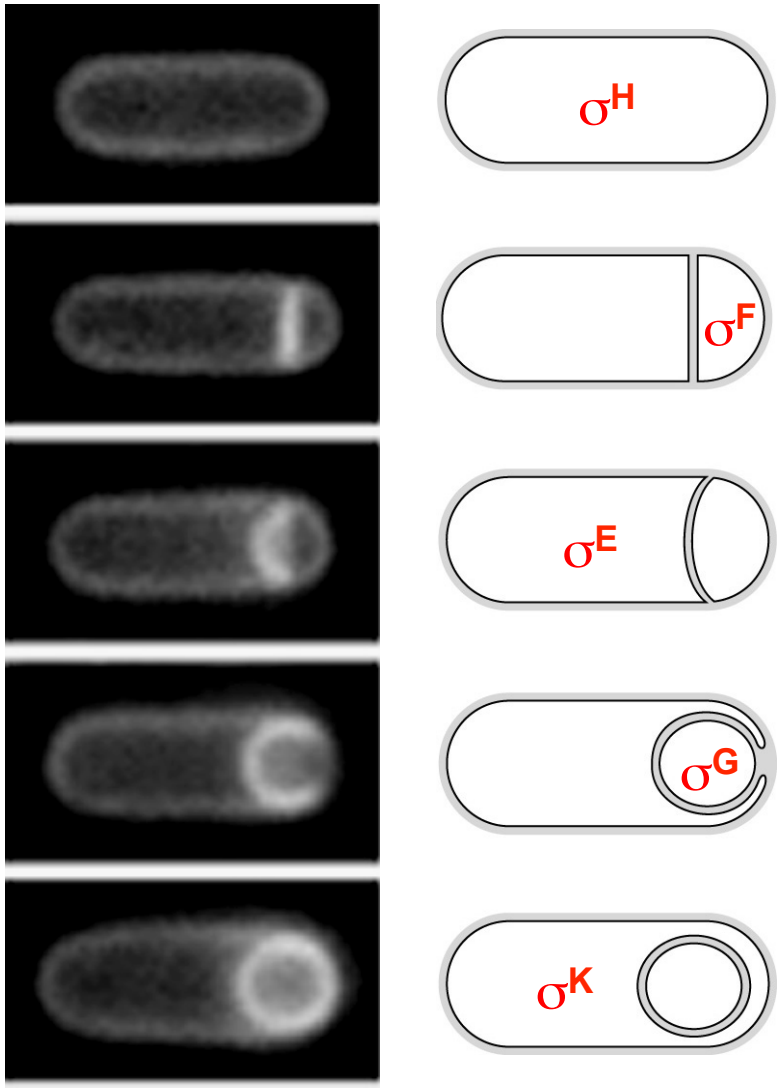
(Prof. of Biology and Applied Physics)

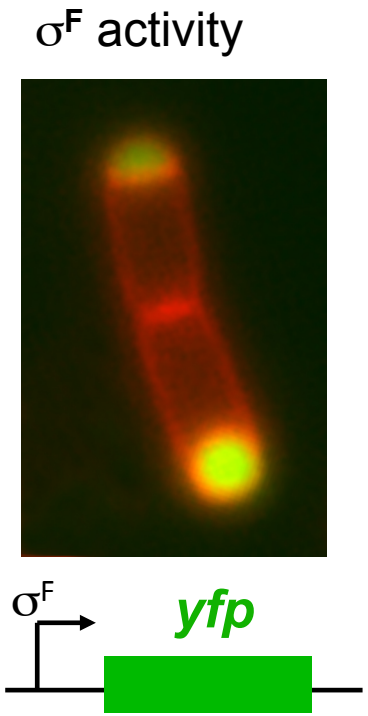
Vegetative growth can turn into sporulation under stressful conditions

Some cells become momentarily competent (transient red color) ; recall last week's lecture!

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!
- Promotor is driving YFP expression (promoter-fusion—allows to track where σF is active)

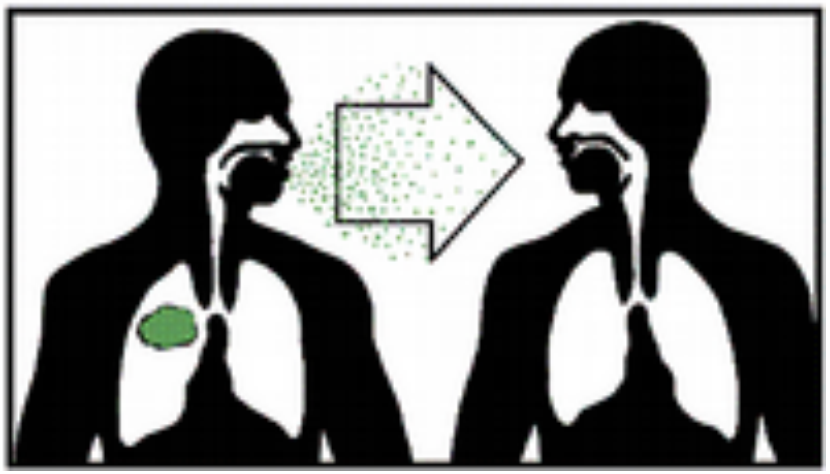
Let’s take a close-up look using cryoEM at sporulation in *Acetonema longum* *(Grant Jensen’s Lab)*:

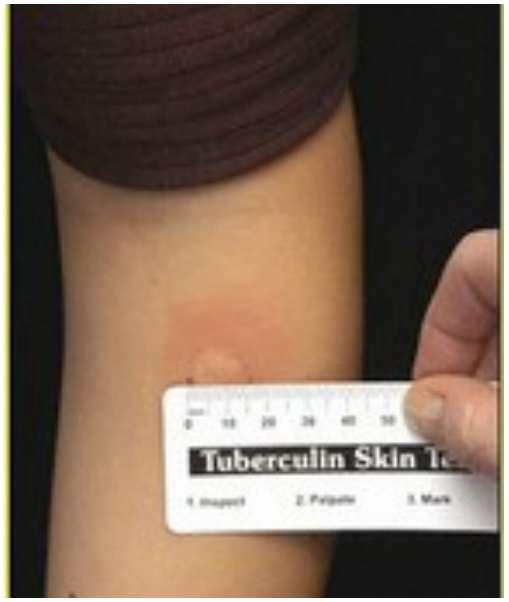
http://lab.jensengroup.org/movies/Acetonema_300MG_final-desktop.mov

What happens when times get tough? Quiessence

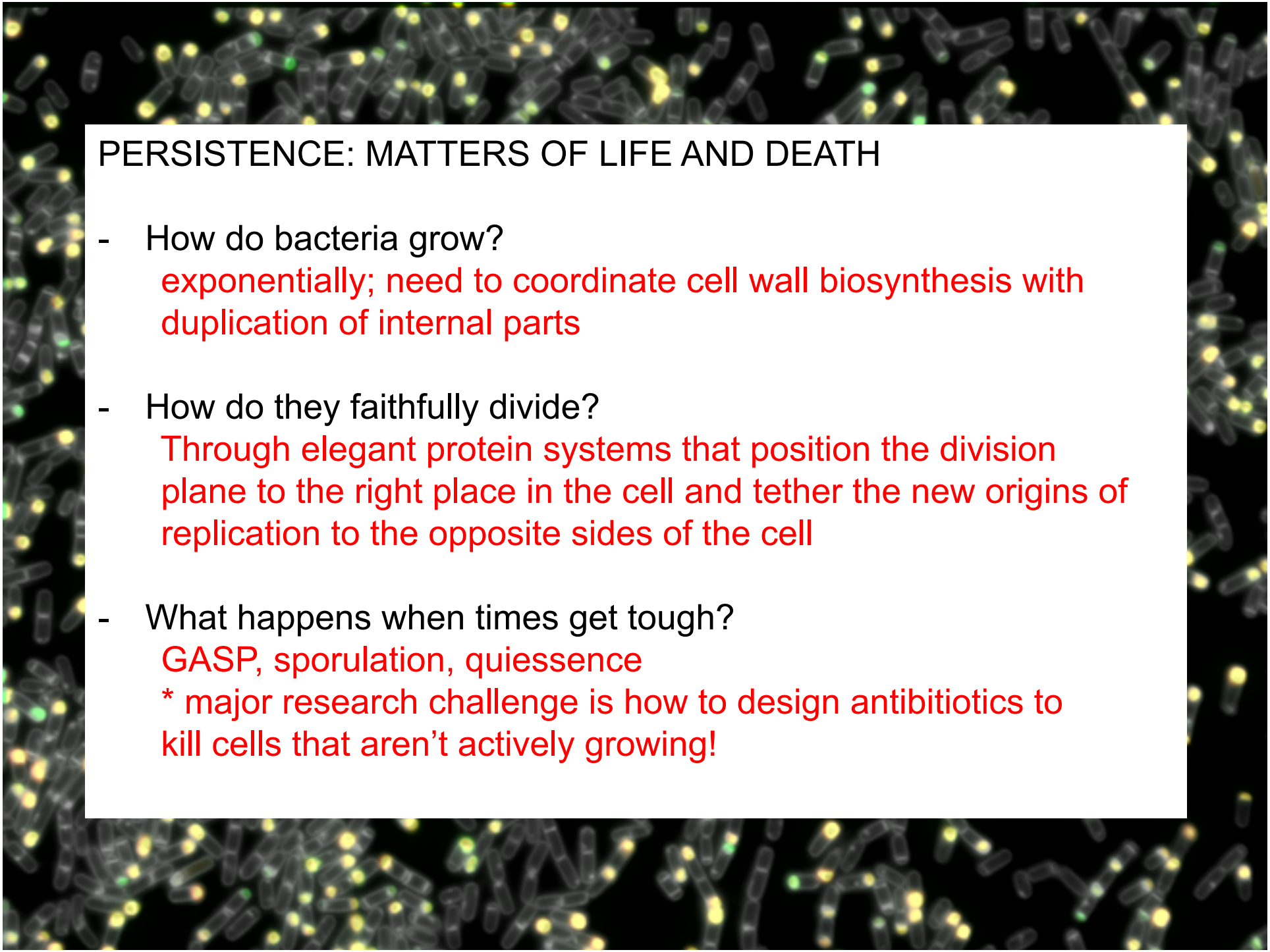
Mycobacterium tuberculosis (M.tb): 1 in 3 people harbor it worldwide

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





- key 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?**

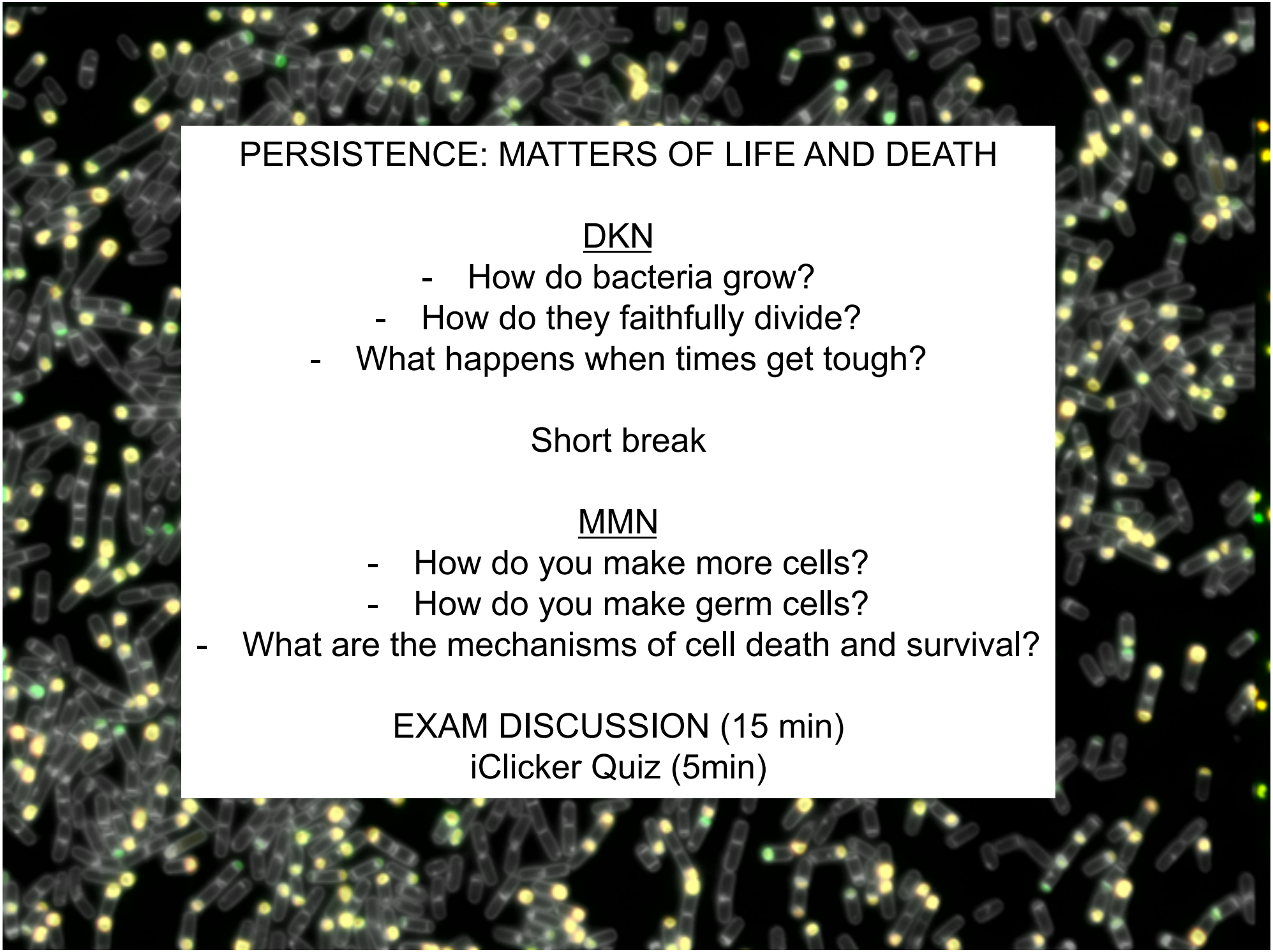


PERSISTENCE: MATTERS OF LIFE AND DEATH

- How do bacteria grow?
  exponentially; need to 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, quiessence
  * major research challenge is how to design antibitiotics to kill cells that aren't actively growing!



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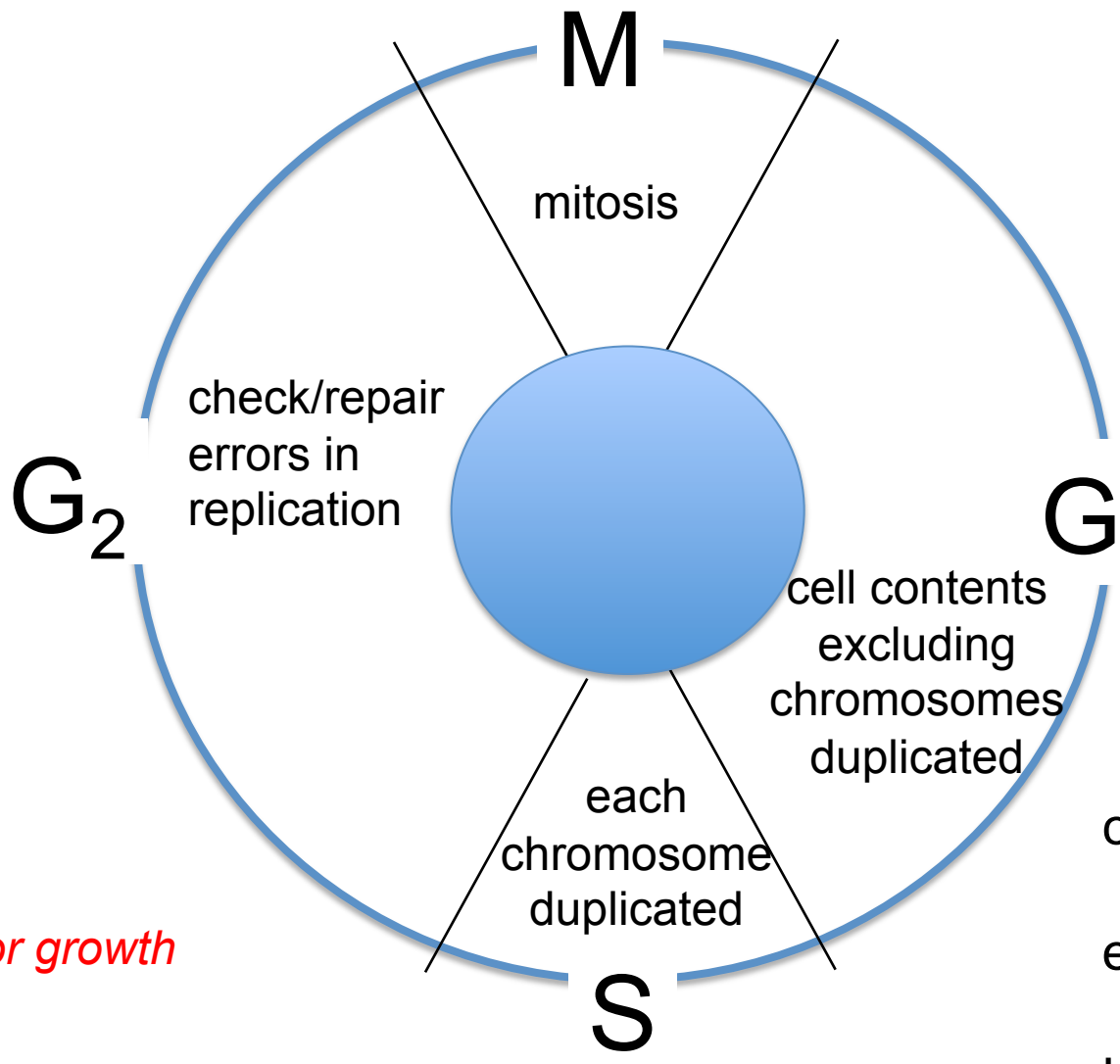
EXAM DISCUSSION (15 min)
iClicker Quiz (5min)

The Lives of a Cell

Birth – Growth – Function – Division - Death

Cell Cycle

M = mitosis



G = gap or growth

S = synthesis

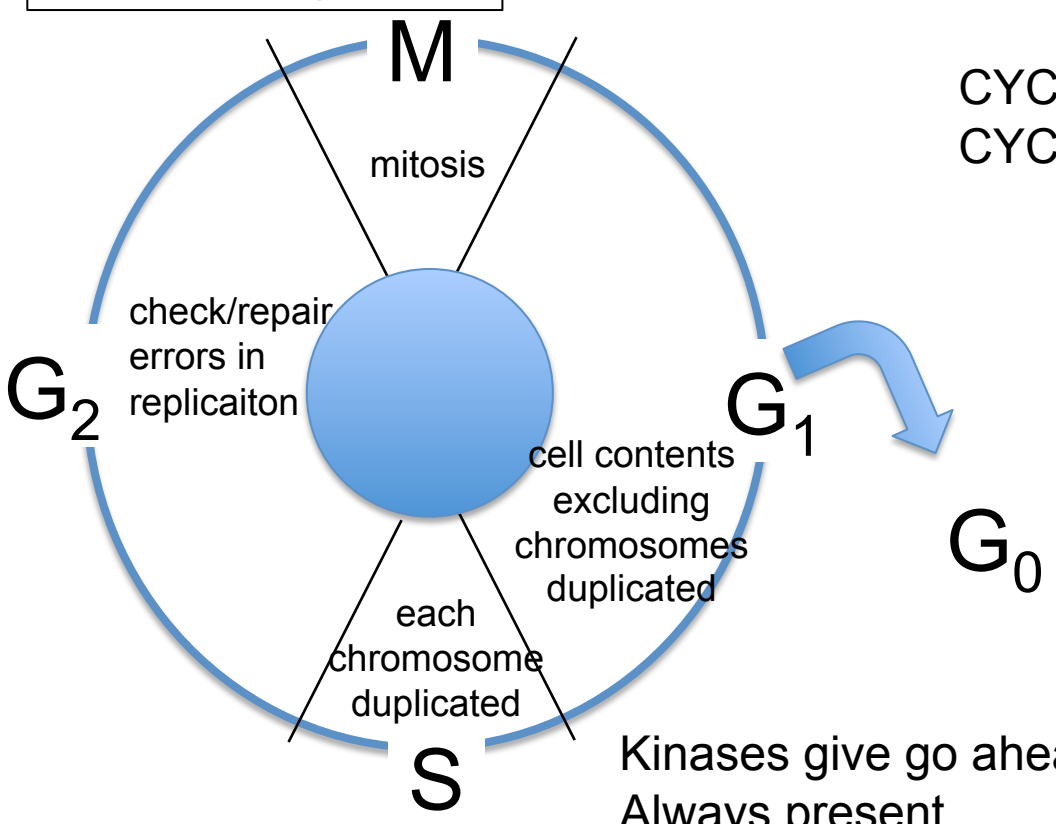
G_0

cell cycle arrest

e.g.?

Leave G_o? - Cancer

Cell Cycle



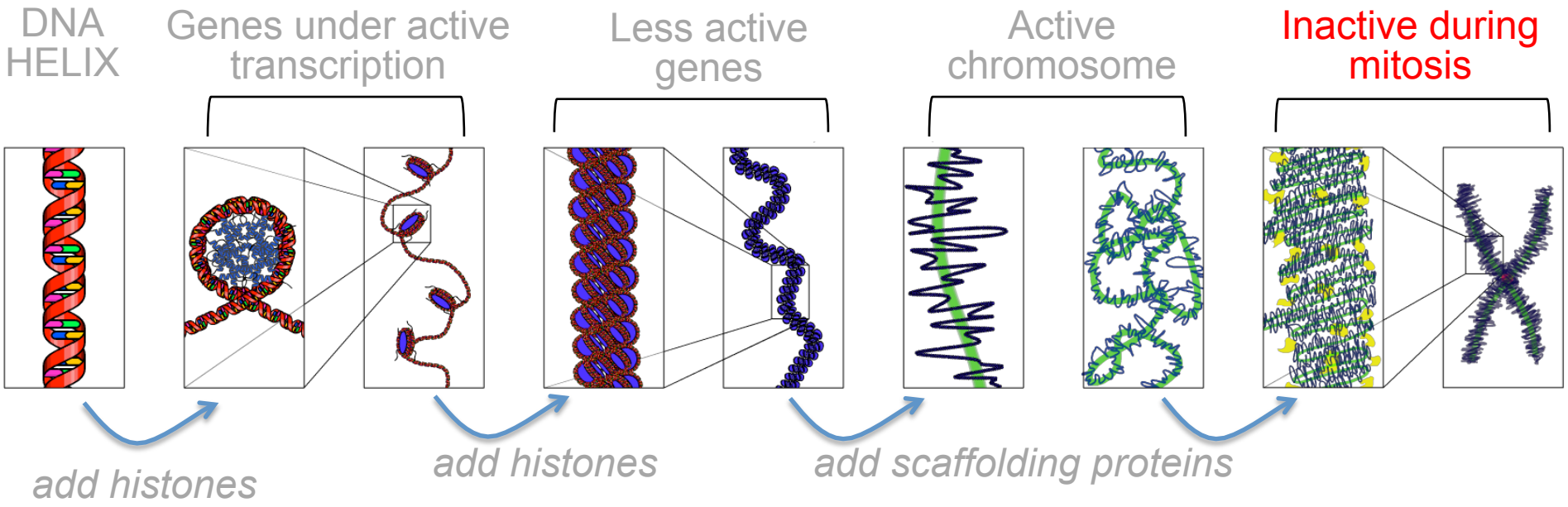
Regulation by two protein types:

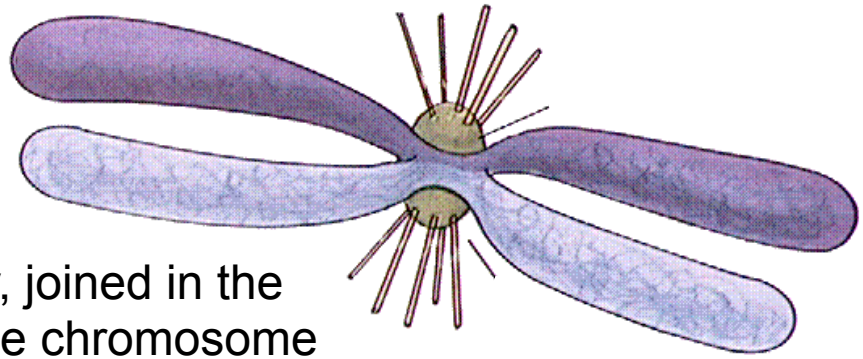
CYCLINS
CYCLIN-DEPENDENT KINASES (CDKs)

Kinases give go ahead signal for cycle advancement
Always present
Must be bound to cyclin to be active
Different cyclins are active, depending on position in cell cycle – when high, bind kinases, which promote progression

P53 tumor suppressor – indirectly inhibits protein CDKs of G1 – cell cycle arrest (repair or cell death) *Science* molecule of the year 1993

Levels of Organization of the Chromatin/ Chromatin Packing:

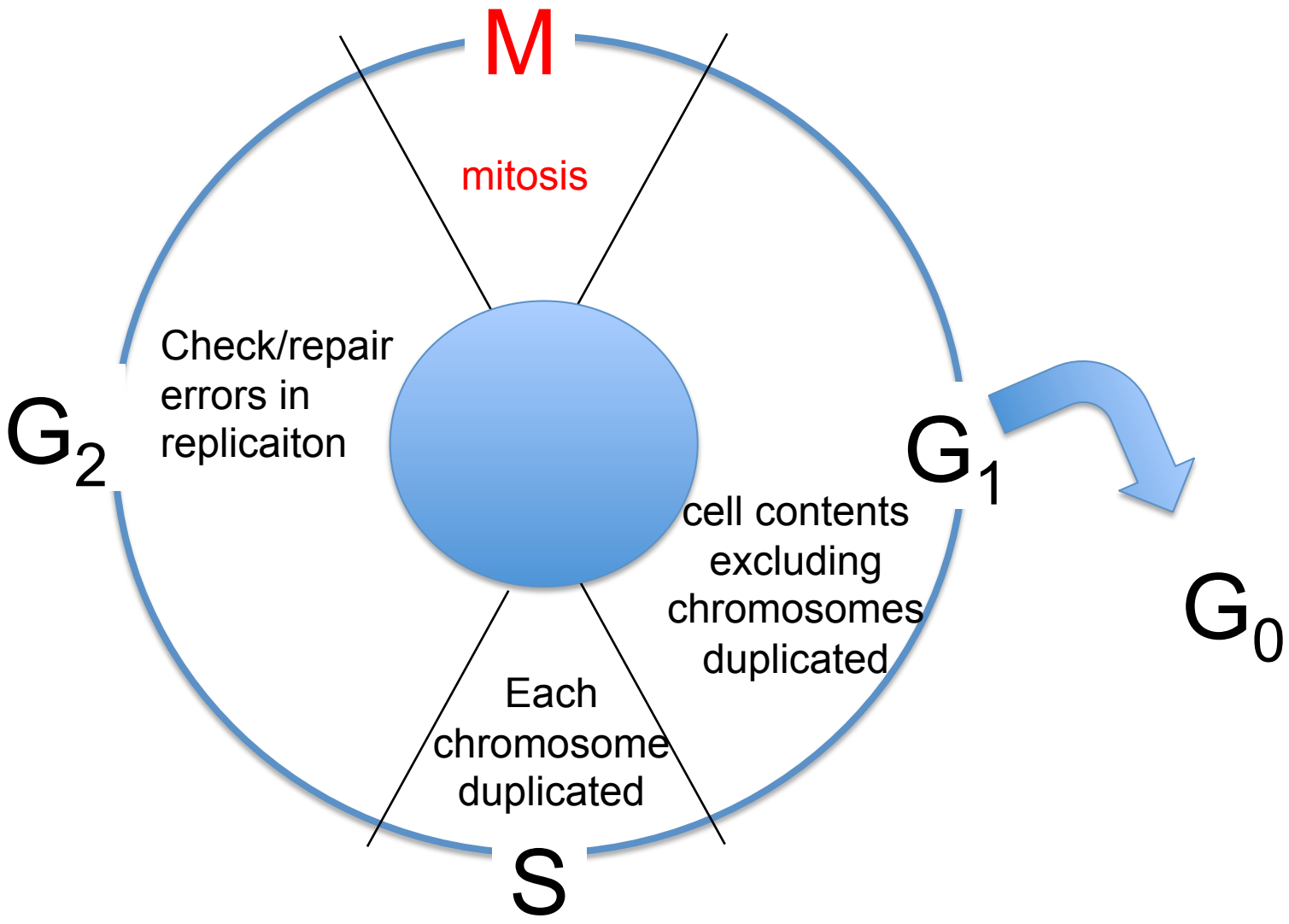




daughter chromatids

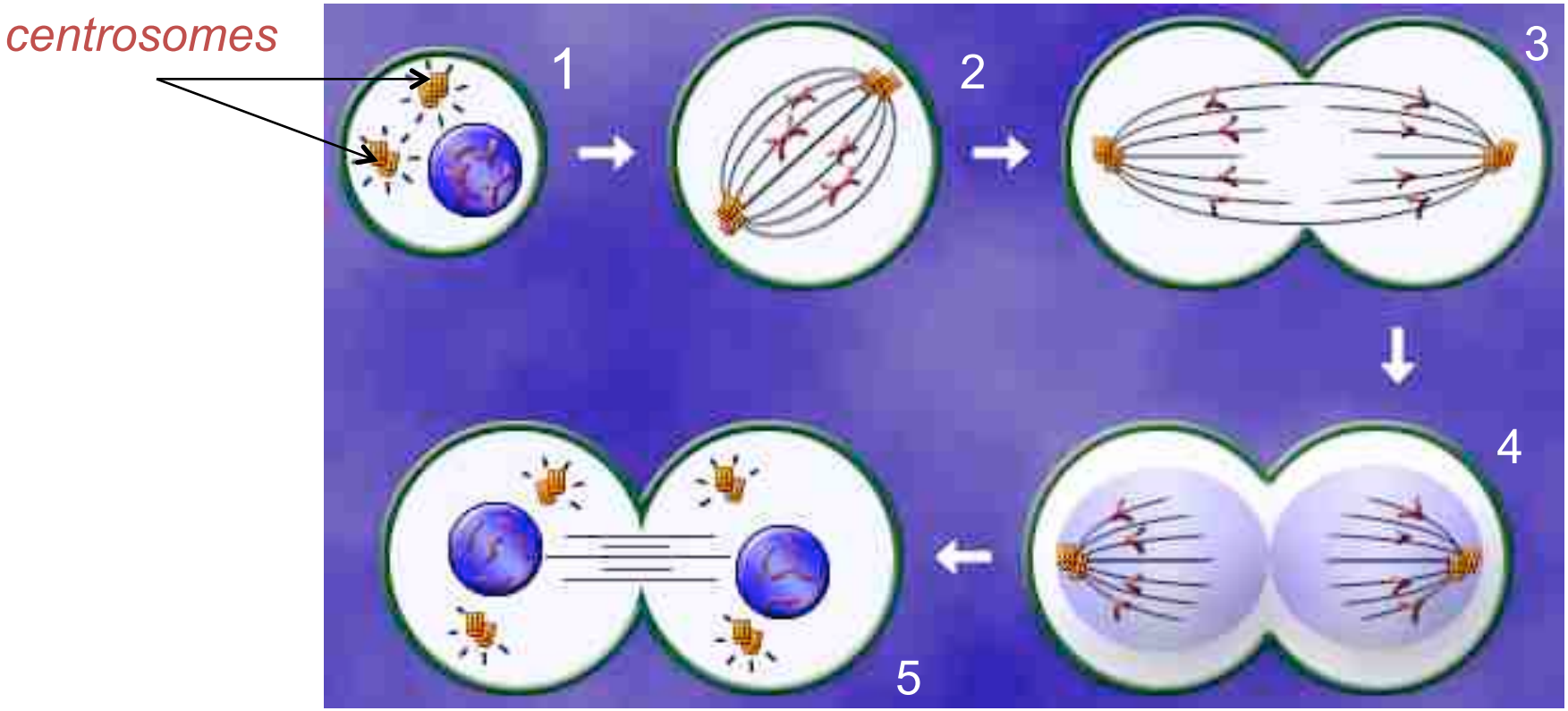
After S-phase-
chromosome has a duplicate copy, joined in the center (~100 proteins involved); the chromosome with the two daughter chromatids are condensed like this during early M phase

Mitosis – cell division, maintaining the same chromosome number (usually diploid, or 2n)



Mitosis

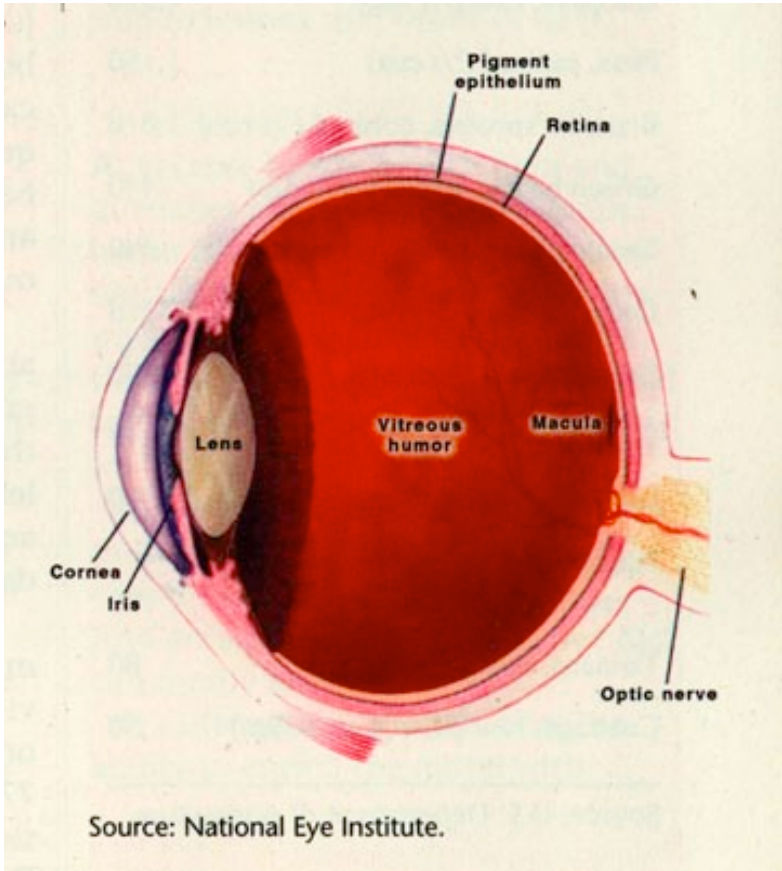
Stage 1 – chromatin condenses, centrosomes formed and go to opposite sides of the cell
Stage 2 – nuclear membrane lost, chromosomes line up along the center, fibers from centrosomes attach to protein complex holding chromatids together
Stage 3 – fibers pull chromatids apart and to opposite poles
Stage 4 – nuclear membrane begins to reform
Stage 5 – nuclear membrane formed, protein fibers disappear
- Then cell completes division to make two cells



Human body
>200 cell types:

Lifespans –

white blood cells – 6-7 h; RBC 120 d
stomach-lining cells 2 d
bone cells 25-30 y
lens cells epithelium/fiber cells



Plants and Animals have another type of division:

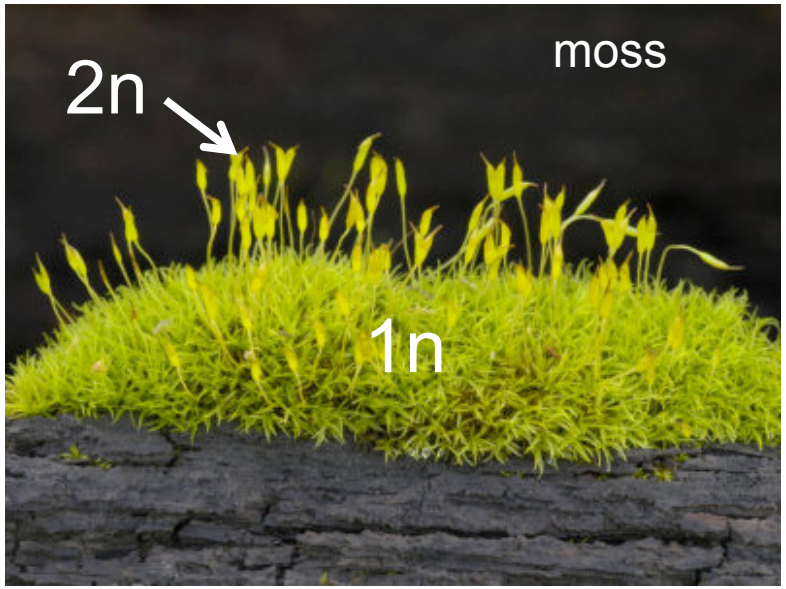
MEIOSIS = reduction division, as you end up with ½ the number of chromosomes at the end of the process (often 2n reduced to 1n)

In plants often:

mitosis (2n) – Meiosis 1n – mitosis to produce 1n body – some 1n cells specialize to become egg (1n) and sperm (1) – fertilization (2n)
Many mitoses to become (2n plant)

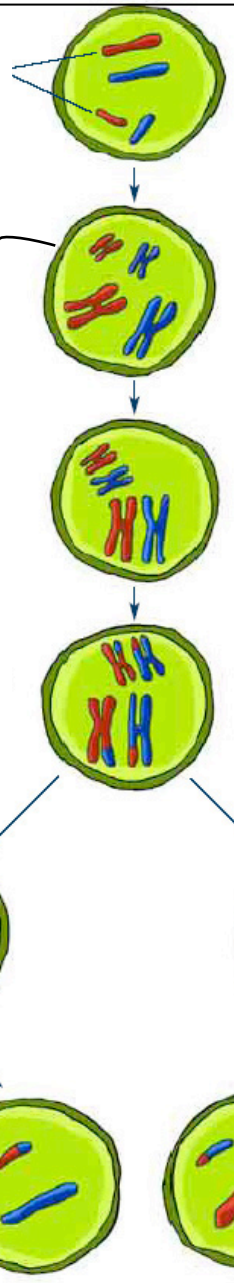
Plant evolution

dominance of 1n ⟶ dominance of 2n



In animals: Meiosis typically makes gametes

chromosomes from parents (one from each)



Cell nucleus of 1 cell - 2n

Meiosis 1 –

A. homologous chromosomes come together and exchange genetic material

B. Homologous chromosomes segregate into two daughter cells (2n to 1n)

Meiosis 2 –

A. Like mitosis, chromatids segregate into

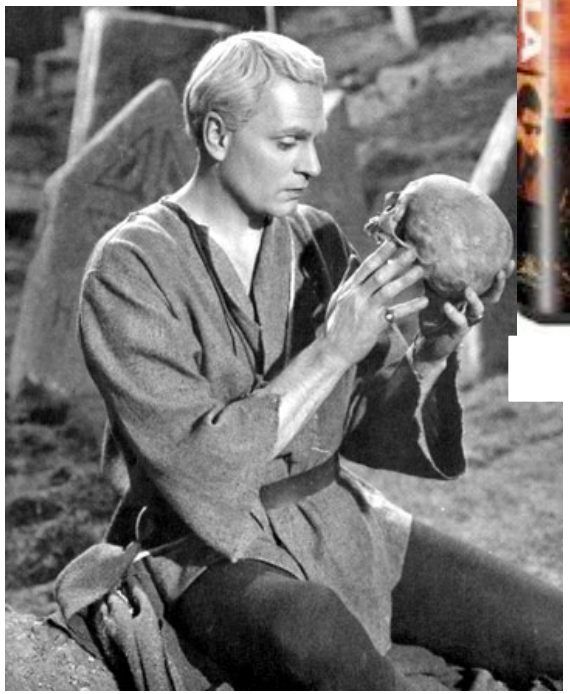
Cell nuclei of 4 daughter cells - 1n

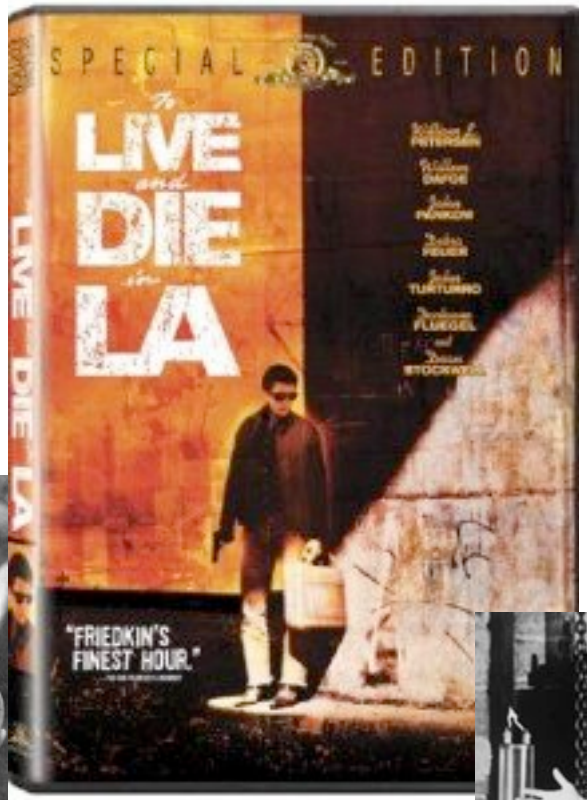
Barbara McClintock
(1902-1992)

Discovered crossing over during meiosis 1

Nobel Prize in Physiology/Medicine 1983

To Live or Die? Apoptosis/Autophagy



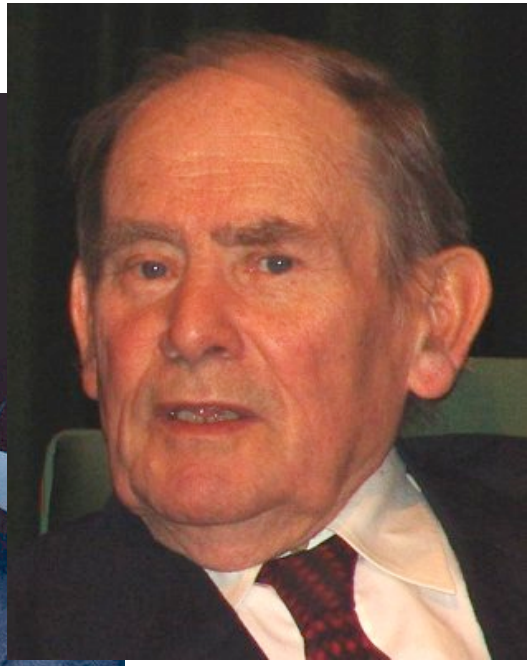


Apoptosis – Programmed Cell Death

Nobel Prize in Medicine 2002

Robert Horvitz - MIT

Sir John Sulston
Wellcome Trust/Sanger Inst



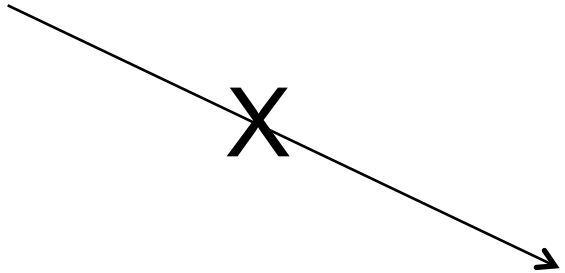
Sydney Brenner
UC Berkeley

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

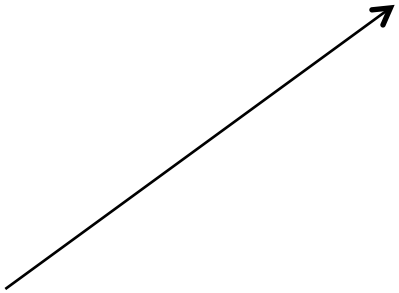
<u>Apoptosis</u>

Tightly controlled death

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



INFLAMMATION

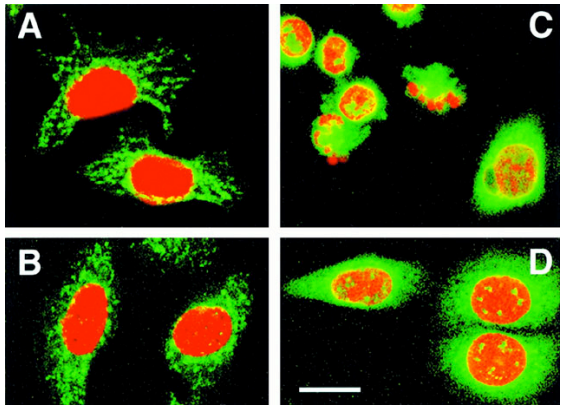


Necrosis

Uncontrolled death & destruction

Characteristics of Apoptosis

- Early - Mitochondria targeted - Increase in mitochondrial membrane permeability

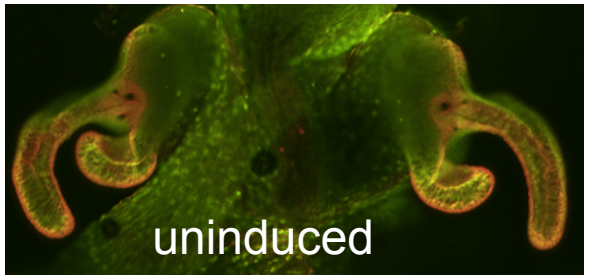


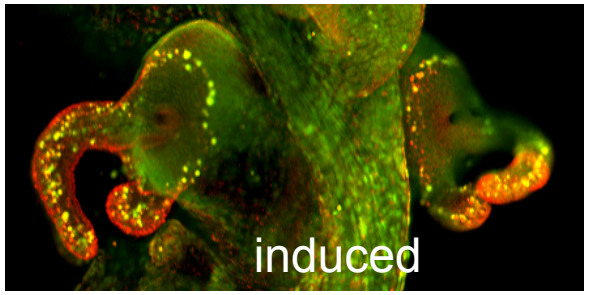
Uninduced Induced

Cytochrome c of mitochondria released into the cytoplasm [nuclei]

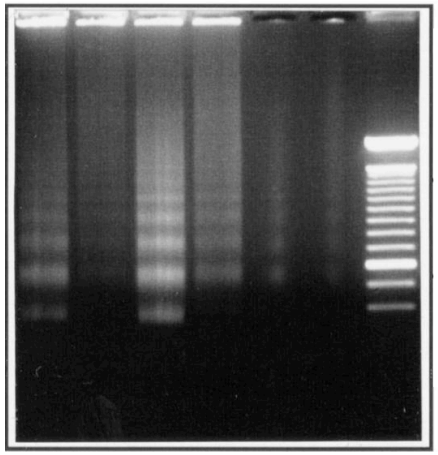
?

-Cells shrivel/round
chromatin condenses/DNA fragments





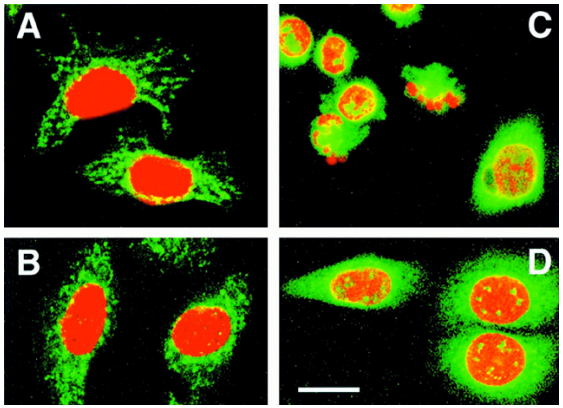
Condensed chromatin



DNA fragmentation into ~180 bp bits

Steps in Apoptosis

- Mitochondria targeted - Increase in mitochondrial membrane permeability



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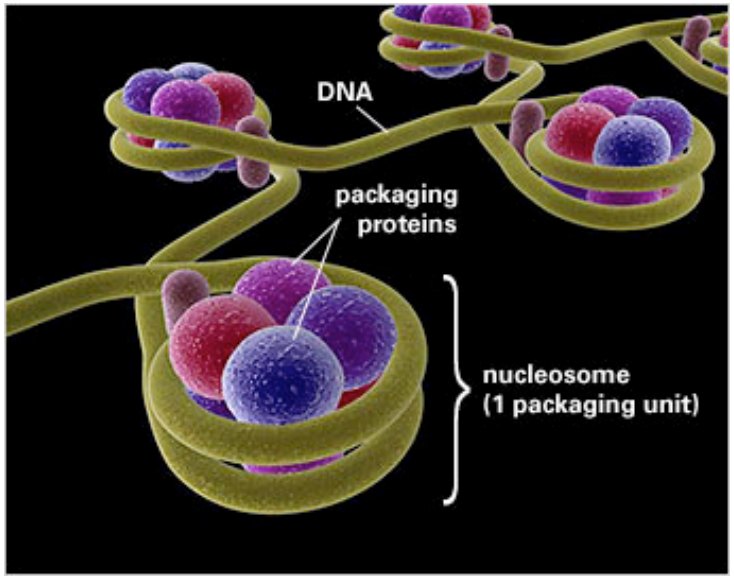
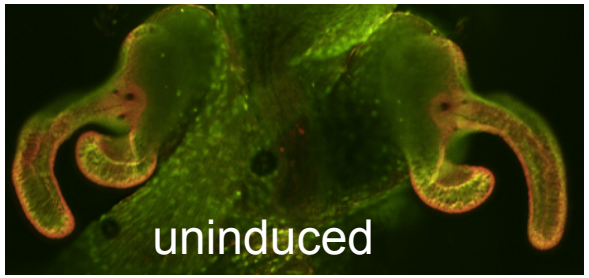
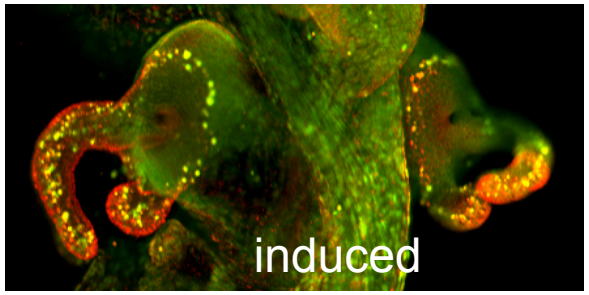


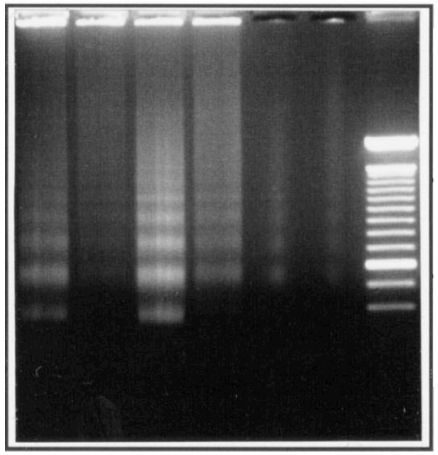
?

-Cells shrivel/round
chromatin condenses/DNA fragments



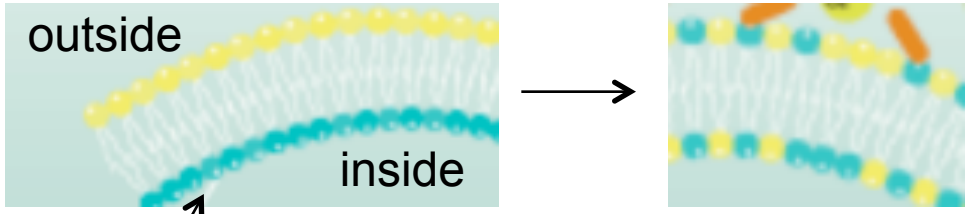


Condensed chromatin



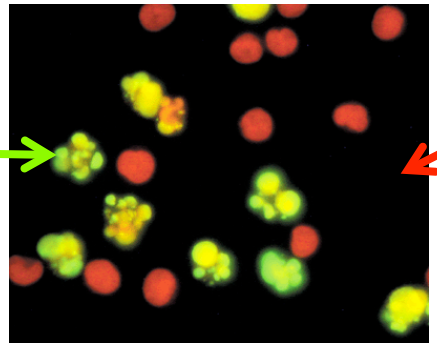
DNA fragmentation into ~180 bp bits

Surface/shape changes in apoptosis

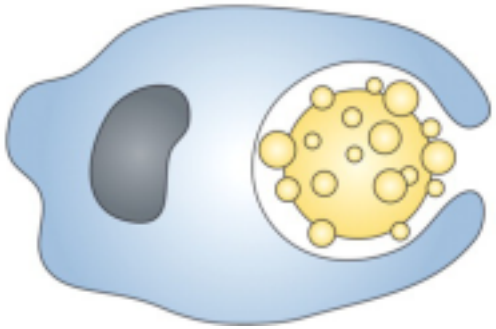


phosphatidylserine

Membrane polarity compromised



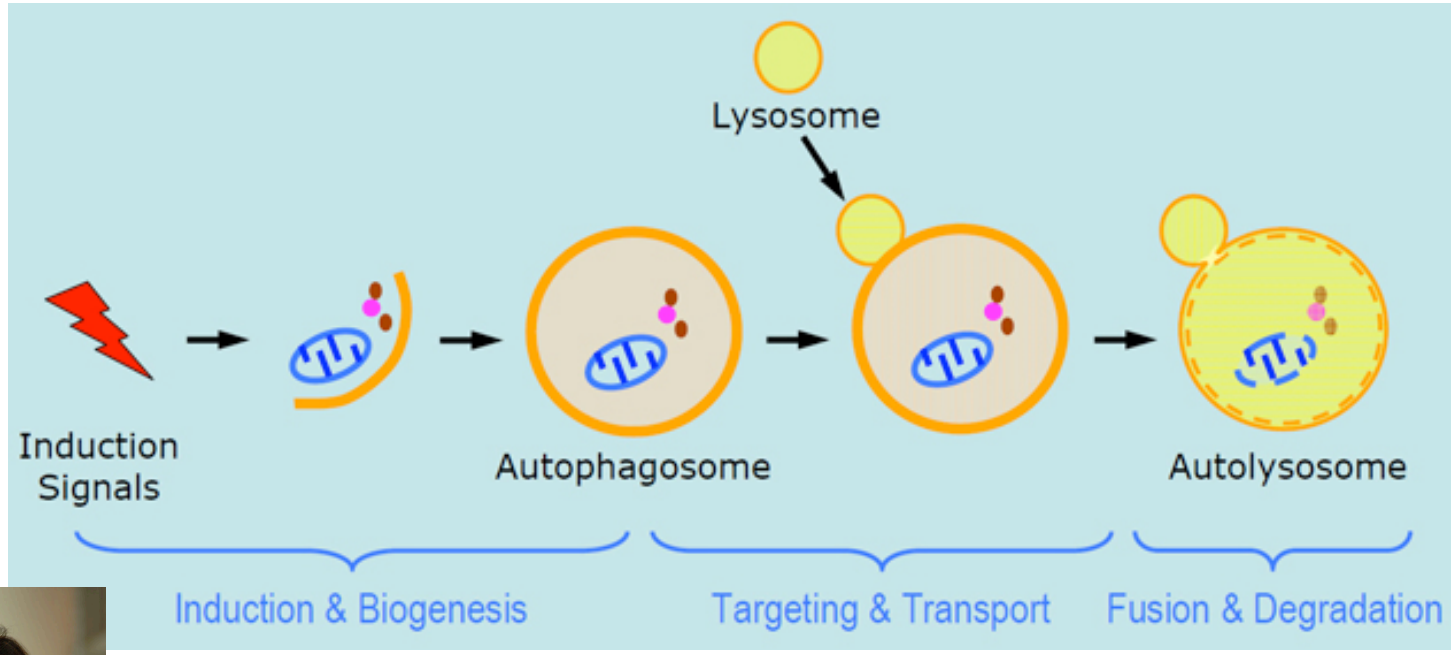
Blebbing, production of apoptotic bodies



Phagocytosis of the corpse

Alternative pathway -

Autophagy: degradation of a cell's components by its own machinery; maintains the balance between synthesis, degradation and recycling of cell material



THE MEMBRANES OF THE AUTOPHAGOSOME COME MITOCHONDRIAL MEMBRANES!!

Jennifer Lippincott-Schwartz
National Institutes of Health

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