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?
\[ y = 2^x \]

\( 
\rightarrow E. \text{ coli (20 min gen time)} \\
< 2 \text{ days to take over the Earth!} 
\)

*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. magneticum

**Vibrio**
- V. harveyi

Example

→ Do they always have the same shape?
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 leads to lysis.

E. coli or B. subtilis depleted leads to lysis.
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 (elongation)
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 charm 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?

2 nucleoids

Cell wall/membrane

Origin of replication

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

→ See Prof. Richard Losick’s *iBioseminar* 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

Phase contrast

DAPI-stains DNA

FtsZ

FtsZ + DAPI

growing (G2)
division
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

1 day old
3 day old
10 day old
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 *Acetonema longum* (Grant Jensen’s Lab, Caltech):

http://lab.jensengroup.org/movies/Acetonema_300MG_final-desktop.mov
What happens when times get tough? 

*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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The Lives of a Cell

Birth – Growth – Function – Division - Death
Cell Cycle

- M: Mitosis
- G1: growth (gap)
- G0: arrest
- G2: check repair
- S: synthesis
- Leave G0 → Cancer
Cell Cycle

- G₂: Check/repair, errors in replication
- G₁: Each chromosome duplicated
- S: Cell contents excluding chromosomes duplicated
- M: Mitosis

Regulation:
Cdcin-depended kinases (CDKs)

p52: Tumor suppressor - cell cycle arrest

Science molecule of the year 1993
Levels of Organization of the Chromatin/Chromatin Packing:

- DNA Helix
- Genes under active transcription
- Less active genes
- Active chromosome

1. Add histones
2. Add histones
3. Add scaffolding proteins

Inactive during mitosis

Sphere

~100 proteins
2 daughter chromatids
Mitosis – cell division, maintaining the same chromosome # (2n) diploid
MITOSIS

1. Chromatin condenses

centrosomes

nuclear membrane lost

G_{1}

microtubules

1 → 2 → 3 → 4 → 5
Human body
>200 cell types:

Lifespans –

* White blood cells: 6-7 hrs
* Red blood cells: 120 days
* Bone cells: 25-30 yrs
* Lens

Source: National Eye Institute.
Plants and Animals have another type of division:

MEIOSIS = reduction division  \(2n \rightarrow 1n\)

In plants often:

- gene expression results from mitosis
- genes are produced and result from meiosis

Plant evolution

- dominance of \(1n\) \(\rightarrow\) dominance of \(2n\)

- \(2n\) moss
- angiosperms

Everything you see is \(2n\)
Meiosis

chromosomes from parents (one from each)

Meiosis 1
A. homologous chromosomes come together and exchange parts
B. segregate into 2 daughter cells

Meiosis 2
like mitosis

Cell nucleus of 1 cell - 2n

Cell nuclei of 4 daughter cells - 1n

mitochondria
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
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

Cells shrivel/round chromatin condenses/DNA fragments

Condensed chromatin

Cytochrome c of mitochondria released into the cytoplasm [nuclei]

DNA fragmentation into ~180 bp bits
Steps in Apoptosis

- Mitochondria targeted - Increase in mitochondrial membrane permeability

![Uninduced](A) ![Induced](B)

Cytochrome c of mitochondria released into the cytoplasm [nuclei]

- Cells shrivel/round chromatin condenses/DNA fragments

![uninduced](images/uninduced.png)

![induced](images/induced.png)

Condensed chromatin

DNA fragmentation into ~180 bp bits
Surface/shape changes in apoptosis

outside → inside

phosphatidylserine

Membrane polarity lost

Living cell

Blebbing

Phagocytosis of the corpse
Alternative pathway -

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

Induction & Biogenesis  Targeting & Transport  Fusion & Degradation

The membranes of autophagosome come from autochondrial 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
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.*