Cell Cycle Lectures

L1: Introduction to the Cell Cycle

L2-4: Cytoskeleton Structure and Dynamics

L5-7: Chromosomes in the Cell Cycle

L8: Cell Cycle Control

L9: Cell Cycle Checkpoints

L10: The Cell Cycle and Cancer
Lecture 1
Introduction to the Cell Cycle

Outline:
Cell cycle basics
Interphase and mitosis
Cell cycle regulation
Variant cell cycles
Consequences of defective cell division
Methods for studying the cell cycle

Paper: check the website tonight

Paul Nurse “Controlling the Cell Cycle” !!!
Thu 4 PM, 100 GPBB
Cell Cycle Basics

sequence of events for one round of cell duplication

great example of how cells integrate internal and external signals and structures to accomplish a dynamic, essential function

very complex, but significant knowledge generated over past 20 years
Cells spend most of the time in interphase

sit around, looking bored and boring...but...
Interphase

- **G1**: cell growth, gene expression
- **S**: DNA replication, gene expression
- **G2**: cell growth, prepare for mitosis

**Microtubules**

**DNA**
Reason for mitosis - accurate segregation of replicated DNA

.....requires exquisite, dynamic coordination with cytoskeleton & other cellular components
Mitosis: Dynamic Cytoskeletal and Nuclear Events

**nuclear envelope:**
breaks down and reforms

**nucleolus:**
breaks down and reforms

**chromosomes:**
replicate, condense, segregate

**centrioles:**
duplicate and separate

**microtubules:**
radial array becomes bipolar spindle

**actin:**
cytokinetic furrow formation
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<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>A. Interphase</td>
<td>B. Prophase</td>
<td>C. Prometaphase</td>
<td>D. Metaphase</td>
</tr>
</tbody>
</table>
|   | Nucleus | Centrosome | Nuclear envelope (NE)  
Centromes separate  
Chromosomes condense  
Chromosomes attach to spindle  
Chromosomes align on spindle equator |
| Microtubules |   |   |   |
|   |   |   |   |
| E. Anaphase A | F. Anaphase B | G. Telophase | H. Cytokinesis |
|   |   |   |   |
| Sister chromatids separate and move to poles  
Cleavage furrow (CF) assembles  
Organized central spindle (CS) assembles  
Poles (arrows) separate  
Cleavage furrow (CF) constricts  
Nuclear envelope (NE) reassembles  
Chromosomes decondense  
Interphase microtubule network reforms  
Daughter cells separate |   |   |   |
prophase
prometaphase
metaphase
Drosophila tissue culture cell in mitosis

Histone-GFP
Tubulin-RFP
How do we know cell division is regulated?

- Growth factors
- Density-dependent inhibition
- Anchorage dependence

300 million new cells in our bodies daily!
Flu virus or polyethylene glycol

Human cells

Chicken cells

Homokaryon

Human cells

Homokaryon
S phase cells

G₁ phase cells

induced to replicate DNA
Mitotic
cell

G1, S, or G2
interphase cell

Homokaryon

Heterokaryon

Homokaryon
why do these chromosomes look so crappy?
Experiment 1

S phase ‘cytoplasm’ dominant over G1

Experiment 2

M ‘cytoplasm’ dominant over interphase
G1/S is the major point of regulation

environment, cell size
(nutrients, contact with other cells, extracellular signals)
Cell Death is an Integral Part of Cell Cycle Regulation

**Apoptosis** - programmed cell death, ‘suicide’

**Necrosis** - not programmed, eg due to injury
Necrosis
‘The Departed’

DiCaprio

Damon

HMGB1-YFP in HeLa Cells (UV-treated)

Time-lapse interval = 2 mins
Frame rate = 10 fps

HeLa Cells, UV damage
Apoptosis and Development

- Sculpting of embryonic forms
- Development of nervous system
- Elimination of self-reactive parts of the immune system

Interdigital cell death elaborates digits

Apoptosis eliminates a tadpole’s tail
Programmed Cell Death in Human Development

- Epithelial cells must die to allow fusion of palate
- Mammary epithelium cells die when deprived of hormones at end of lactation
- Cells of müllerian ducts die in males
- Prostate cells die when deprived of hormone
- Cells of interdigital webbing die
- Up to 80% of neurons die in some ganglia
- Over 95% of immature T cells die in thymus
- Dying cells (yellow)
Apoptosis Required for Normal Development

Normal Apoptosis

Dysfunctional Apoptosis
Cell death is essential during formation of the nervous system.
Variations on a Theme.. Many Types of Cell Divisions

**Somatic cell cycles** - slow (~24 hrs), have G1 and G2

**Embryonic cell cycles** - fast, only S & M

**Drosophila**

6-10 minute cycles, synchronous nuclear division in one cell (syncytium)

DNA

Tubulin
Variations on a Theme.. Many Types of Cell Divisions

**Embryonic cell cycles** - fast, only S & M

Vertebrates, Sea Urchins

30-60 minute cycles, cell divisions
Variations on a Theme.. Many Types of Cell Cycles

Polyploidy - multiple copies of normal genome (e.g., 4 instead of 2)

occurs normally, e.g., liver cells hexaploid

Endoreplication - continued replication and growth without division
Variations on a Theme.. Many Types of Cell Cycles

**Endoreplication** - continued replication and growth without division

**Polyteny** - homologous chromosomes retain alignment
Variations on a Theme.. Many Types of Cell Cycles

Asymmetric divisions - unequal segregation of cell fate determinants

Stem cells
Variations on a Theme.. Many Types of Cell Cycles

Asymmetric divisions - unequal segregation of cell fate determinants

Neuroblasts

Miranda maintains undetermined state
Variations on a Theme... Many Types of Cell Cycles

Meiosis - production of haploid germ cells

<table>
<thead>
<tr>
<th>MITOSIS</th>
<th>MEIOSIS</th>
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<tbody>
<tr>
<td><strong>Prophase</strong></td>
<td></td>
</tr>
<tr>
<td>Duplicated chromosome (two sister chromatids)</td>
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<tr>
<td>Chromosome replication</td>
<td></td>
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<tr>
<td>Parent cell (before chromosome replication)</td>
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<tr>
<td>Chiasma (site of crossing over)</td>
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<tr>
<td><strong>Parent cell</strong></td>
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<tr>
<td><strong>Prophase I</strong></td>
<td></td>
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<tr>
<td>Tetrad formed by synopsis of homologous chromosomes</td>
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<tr>
<td><strong>Metaphase</strong></td>
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<tr>
<td>Chromosomes align at the metaphase plate</td>
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<tr>
<td><strong>Metaphase I</strong></td>
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<tr>
<td><strong>Anaphase I</strong></td>
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<tr>
<td><strong>Telophase I</strong></td>
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<tr>
<td>Homologous chromosomes separate during anaphase I; sister chromatids remain together</td>
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<tr>
<td><strong>Daughter cells of meiosis I</strong></td>
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<tr>
<td>Haploid</td>
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<tr>
<td>n = 2</td>
<td></td>
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<td><strong>Daughter cells of meiosis II</strong></td>
<td></td>
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<tr>
<td>No further chromosomal replication; sister chromatids separate during anaphase II</td>
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</table>

Daughter cells of mitosis | Daughter cells of meiosis I | Daughter cells of meiosis II
Consequences of Defective Cell Divisions

unrestricted cell growth & cancer

chromosome missegregation & aneuploidy

loss of DNA integrity
seed new culture, growth and division
‘anchorage dependent’

cells fill plate, stop growth and division
‘contact’ or ‘density-dependent’ inhibition

remove cells
new round of growth

stop growth and division

(a) Normal mammalian cells
Chromosome non-disjunction: aneuploidy

→ TOAST
Karyotype of malignant pancreatic tumor

defects in chromosome segregation:
→ birth defects
→ associated with tumor progression
chromosome condensation during S-phase

→ TOAST

DNA damage:
chemicals
radiation
normal DNA metabolism
Methods for Studying the Cell Cycle

cell fusion
live and fixed imaging

genetics
biochemistry
in vitro systems

inhibitors of cellular processes
(transcription, replication, microtubules)
Cell Sorting

sort cell cycle stages based on total amount of DNA
cells in G1 phase

number of cells

relative amount of DNA per cell (arbitrary units)

cells in G2 and M phases

cells in S phase
Synchronizing Cells by Replication Inhibition

DNA synthesis inhibitor
(time greater than $G_2 + M + G_1$)

Release these cells from inhibition, and count cell number
Synchronizing Cells by Replication Inhibition

add BrdU to determine which cells are in S phase, and how much DNA is replicated

no synchronization

after synchronization
Synchronizing Cells by Mitotic Inhibition

colchicine / colcemid / nocodazole - microtubule assembly inhibitors
taxol - microtubule disassembly inhibitor

+ nocodazole

prometaphase arrest

Lib.

+nocodazole, shake off & transfer
Genetic Screens: Yeast ‘Cell Division Cycle’ (CDC) Mutants

‘Dominos’ sequential, dependent events

‘Oscillator’ central controller

Can individual protein mutations block steps or whole process?

• Lee Hartwell (cerevisiae); Paul Nurse (pombe)
• **Goal:** find mutants unable to transit the cell cycle
• **Why yeast?**
  - Cell shape --> cell cycle stage
  - Grow as haploids (easier to find mutants), or diploids (can do genetics)
• **Problem:**
  - the screen is for cells that can’t grow
• **Solution:**
  - temperature sensitive mutants
  - Replica plating
In vitro Dissection of the Cell Cycle - Xenopus Egg Extracts

use to isolate proteins present at particular stages
manipulate proteins-deplete and observe changes to cell cycle
Thursday

L2-4: Cytoskeleton Structure and Dynamics