Cell division control is critical for both proper Development and Guarding against Cancer.

Timing - clock
Clock changes impact output
Different portions of the cell cycle (clock) are altered to produce different outcomes.

**Growth without division**
- oocyte grows without dividing (months)

**Division without Growth**
- fertilized egg divides without growing (hours)
  - sperm
  - egg
  - tadpole feeds, grows, and becomes an adult frog
4 Stages of the cell cycle:

How do we know what we know and how do we measure?

Simple microscopy
Mitosis in a Newt Lung Epithelial Cell

L. Cassimeris and E.D. Salmon

University of North Carolina
Mitosis: condensed chromosomes, NEB and microtubule spindle, chromosome segregation
M-phase: an easy phase to measure...
G1, S or G2 . . . ?
Proportions? What are the relative contributions of each phase to the cell cycle . . ?

S-phase – Chromosome duplication -> produce 2 identical sister chromatids

How do we measure?
Vertebrate cells analyses

H\textsuperscript{3}-thymidine

Relative contributions to Interphase . . . S-phase
Vertebrate cells analyses

$H^3$-thymidine

Bromo-deoxyuridine (BrDU) used in place of thymidine

Relative contributions to Interphase ... S-phase
Proportions? What are the relative contributions of each to the Cell Cycle . . .

How do we measure G1?
DNA content can reveal the G1 portion of cell cycle

Flow cytometry -
Laser-based inquiry of cells in suspension
DNA intercalating Fluorescent dyes
If you know the duration G1, S and M contributes to a cell cycle . . .

G2 is whatever remains . . .

Arrows . . . Directionality?
Directionality of the cell cycle . . .
How do we know?

3 Possibilities:

1. G1 nucleus will stay G1, S will stay S
2. G1 nucleus will transition to S
3. S nucleus will return back to a G1 state
Directionality of the cell cycle . . .
How do we know?

S-phase promoting activity in ‘S’ cell that triggers DNA replication in G1 cell

G1 cell is competent to enter S
Directionality - clocks go in only one direction

No going in reverse (S-phase cell does not induce G2 cell to re-enter S-phase) -> DIRECTIONALITY!

G1-phase nucleus immediately enters S phase; S-phase nucleus continues DNA replication

You can’t rush S ... cells monitor!
Directionality - clocks go in only one direction

G2 cell can not induce G1 cell to replicate DNA

G2 cell has lost the S-phase promoting activity

Molecular mechanism
For ‘losing’ and ‘gaining’ an Activity . . .
Random mutagenesis allows for identification of genes required for specific cell phase activities.
What gene, when mutated, locks cells in mitosis (or G1 or S or . . )?
Master Regulator of the Cell Cycle:
Cyclin-dependent Kinase (CDK)

Cell cycle progression requires CDK activity

Budding yeast - only 1 CDK = Cdc28

Fission yeast - only 1 CDK = Cdc2

Partners in crime:
Cyclin binds/activates kinase subunit

Hmm . . . how does 1 CDK regulate G1, S, G2, M? A study in yeast . . .
Cyclin-dependent Kinase (CDK)

Cell cycle progression requires CDK activity

Different cyclins -

different substrates
Cyclin-CDK complexes:

<table>
<thead>
<tr>
<th>Phase</th>
<th>Cyclin</th>
<th>CDK</th>
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<tbody>
<tr>
<td>Budding</td>
<td>budding</td>
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<tr>
<td>G1</td>
<td>Cdc28</td>
<td>CLN3</td>
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<tr>
<td>G1/S</td>
<td>Cdc28</td>
<td>CLN1,2</td>
</tr>
<tr>
<td>S</td>
<td>Cdc28</td>
<td>CLB5,6</td>
</tr>
<tr>
<td>M</td>
<td>Cdc28</td>
<td>CLB1-4</td>
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Different flavors of cyclin specify CDK activities through the cell cycle.
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<th>vertebrate</th>
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<tr>
<td>G1</td>
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Different catalytic CDK kinase proteins
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<tr>
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<tr>
<td>M</td>
<td>CDK1</td>
</tr>
<tr>
<td></td>
<td>Cyclin B</td>
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</table>

...And Different cyclins
Low CDK
G1 cells can enter Quiescence (G₀) state

Differentiated cells re-enter the cell cycle via external cues

Understanding cancer involves understanding how cells Re-enter the cell cycle in the absence of proper cues!
G1 (and S) cyclin-CDK expression induced by external cues.

MITOGENS

PDGF
  Platelet-derived growth factor
EGF
  epidermal growth factor
TGF-β
  Transforming growth factor
G1 cyclin-CDK activated by external cues: Mitogens!!

1. Wound/trauma activates platelets -> release PDGF

2. PDGF receptor on neighboring cells activates Kinase signal transduction pathway
G1 cyclin-CDK activated by external cues: Mitogens!!

1. Wound/trauma activates platelets
   -> release PDGF

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3. Kinase turns on transcription factors -> gene expression
G1 cyclin-CDK activated by external cues: Mitogens!!

1. Wound/trauma activates platelets -> release PDGF

2. PDGF receptor on neighboring cells activates Kinase signal transduction pathway

3. Kinase turns on transcription factors -> gene expression

Tada!! The target of Mitogen activation . . .
Actions of MYC:

1. Express G1 cyclins

Get a rise in G1-CDK activity! Enter the cell cycle.
Actions of MYC:

1. Express G1 cyclins

Get a rise in G1-CDK activity! Enter the Cycle . . .

2. Express E2F

Get a rise in S-CDK activity! Continue with cycle!
Imagine unregulated and HIGH levels of Myc

Cell cycling no longer regulated by external cues
Expansion of genes that regulate cell cycle progression:

- Myc proto-oncogene amplification
- homogenously staining regions (HSR)
Expansion of genes that regulate cell cycle progression:

Examples of
Myc proto-oncogene amplification
homogenously staining regions (HSR)
Double minute chromosomes

More MYC -> more expression of Cyclin

less reliance on Mitogens -> unregulated CDK activation!
Cancer pathways:

Oncogene – a stuck accelerator in a Driver’s Ed Car . . .
(2 of everything)

Causes:
mutation that falsely signals mitogen is present
Over-active signaler

HER2 receptor - transmembrane tyrosine kinase (EGF-like Receptor) can be either over-expressed or mutated so no longer needs mitogen for activation.

High levels of Her-2 account for 20-30% of all breast cancers.

Herceptin: Ab that blocks Her2 dimerization.
Cancer pathways:

Cells not only have accelerators . . . they have brakes!
Cancer pathways:

(A) overactivity mutation (gain of function)

normal cell → single mutation event creates oncogene → tumor cell

(B) underactivity mutation (loss of function)

normal cell → mutation event inactivates tumor suppressor gene → no effect of mutation in one gene copy → second mutation event inactivates second gene copy → tumor cell

Tumor Suppressors – Brakes needed to slow car down! – Driver ED car!
Rb binds E2F to keep it INACTIVE
(No E2F . . . No Cyclin E expression)

Mutations in Rb bypass Mitogen dependency
Need to get rid of BOTH Rb copies

Loss of heterozygosity (LOH)
mutation need not be the same!!
Cancer is a multi-hit disease (Driver Ed Car analogy)
At least one Stuck accelerator
Both sets of breaks inoperable

Alcohol, smoking and sunlight
  elevate your mutation rate