Cell Cycle III:

S phase & DNA replication

G₂ phase & G₂-M transition

Mitosis and Cytokinesis

Chapters 42, 43, & 44
## Final Exam: Group Assignment

<table>
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Group Chia-Yuan Chen, Stephen Kustra, William Kowalski, Piyawat Chalermkanjana
Nov-29

Group Emily Fredrich, Stephanie Chang, Kush Mangal
Dec-01

Group Shravya Mukka John Goldman, Aishwarya Sukuma
Dec-06 (may need to be adjusted)

Group Mike McCann, Anupama Kuruvilla, Jacob Sheu
Dec-08 (may need to be adjusted)
Final Exam: Reports

• For each group, the presentation PPT file will serve as the final report.

• Students not presenting should submit a one-page report that consists of two sections
  
  → Section I: critical comments on the paper
  → Section II: your questions
Outline

• DNA replication and its regulation in S phase
• S phase checkpoints and S-G₂ transition
• Overview of G₂ phase
• G₂ phase checkpoints and G₂-M transition
• Mitosis
• Cytokinesis
Overview of S Phase

• Main events:
  - Chromosome replication → DNA replication → Histone protein synthesis
  - Centrosome replication

• DNA replication is performed under precise regulation by a complex macromolecular machinery.

• Checkpoints:
  - DNA damages
  - Stalled replication forks
  - Completion of DNA replication
DNA Replication in S Phase (I)

- DNA synthesis proceeds in a 5' to 3' direction.
- DNA replication starts at origins of replication and proceeds bidirectionally.
- Eukaryotic cells use multiple origins to accelerate replication.
- A licensing mechanism ensures each origin is used once and only once.
DNA Replication in S Phase (II)

- Origins of replication
  - *E. coli*: oriC
  - Budding yeast: ARS (autonomously replicating sequences)
  - Mammalian cells: several known origins; overall much less is known.

- Assembly of prereplication complex before the restriction point provides "licenses" to replication origins.

- An inducer, a combination of kinases (e.g. Cdk2-Cyclin E, Skp2 & Cdc7P), triggers the G₁-S transition and formation of the preinitiation complex.
DNA Replication in S Phase (III)

• Binding of a hexameric Mcm (minichromosome maintenance) to the prereplication complex is critical to the licensing process.

• DNA replication is triggered by an inducer, a combination of protein kinases, especially Cdk2-cyclin E.

• DNA replication are divided into three phases: initiation, elongation, and termination.

• DNA replication is organized based on clusters of replication origins (replication foci; each with ~5-6 replication origins).

• Different domains of the genome are replicated at different time points under precise regulation.
A group of three checkpoints control the progression of the S phase:

- Check for DNA breakages
- Check for stalled DNA replication forks
- Delay cell-cycle until DNA replication is complete
Other Events of S Phase

- Histone protein synthesis
  - Human genome: $3.2 \times 10^9$ bp, 200 bp/nucleosome
    → $3.2 \times 10^9$ nucleosomes
  - Synthesis of histone is substantially enhanced in S phase

- Centrosome replication
Overview of G₂ Phase

• G₂ phase is relatively short. G₂-M transition is rapid.

• G₂-M transition is regulated primarily through Cdk1-cyclin B1.

• Cdk1-cyclin B1 burst is controlled by one stimulatory kinase (CAK) and two inhibitory kinases (Wee1 & Myt1).

• G₂-M transition is triggered by three Cdc25 phosphatases.
G₂ Checkpoints & G₂-M Transition

• G₂ checkpoints monitor
  - DNA damages
  - Completion of DNA replication

• G₂ checkpoints are mediated by ATM/ATR and their downstream molecules.
  → Critical final check before cell division
Major Events in G₂-M Transition

- Cdc25 triggers the activation of Cdk2-cyclin A and Cdk1-cyclinB.

- Events:
  - Higher microtubules dynamics
  - Chromosome condensation.
  - Centrosome migration.

- The complex stimulatory and inhibitory signaling mechanism ensures rapid G2-M transition while offers regulatory options.
Overview of M Phase (I)

- In metaphase cells undergo dramatic and complex changes in its structure and organization. Only apoptosis is comparable.

- Mitosis is the most complex process in the cell cycle.
Overview of M Phase (II)

• Cell division is usually symmetric.

• An important exception is the asymmetric division of stem cells.

• Key regulators of metaphase include Cdk2-cyclin A, Cdk1-cyclin B and APC/C\(^{Cdc20}\).
Prophase (I)

- Prophase is the transition phase of G₂ into mitosis.

- Chromosome condensation
  - H1 and H3 are phosphorylated by Cdk1 and Aurora-B, respectively.
  - Condensin enters nucleus.
  - These activities are not essential for chromosome condensation.

- Disassembly of nucleolus

Prophase (II)

- **Microtubules**
  - Microtubules become more dynamic and much shorter.
  - Increased nucleation at centrosomes.
  - Microtubules become organized into two radial arrays.

- Intermediate filaments and actin disassemble.

- Transcription stops.

- **Intracellular organelles**
  - Golgi and ER fragment.
  - Membrane-mediated events greatly decrease.

- **Cell surface**
  - Endocytosis and exocytosis are suppressed.
  - Surface receptors are internalized.

- **Cell shape becomes rounded.**
Overview of Prometaphase

• Major events

- Nuclear envelope breaks down.

- Capture of chromosomes by MTs.

- Chromosomes establish bipolar attachment at kinetochores.

- Correction of attachment errors under the control of spindle checkpoint molecules.
Prometaphase: Nuclear Envelope Breakdown

- Nuclear membrane bilayers are removed.
- Nuclear pores disassemble.
- Nuclear lamina meshwork disassemble.
- Broken nuclear envelope components are organized differently in different cells.
Prometaphase: Mitotic Spindle Organization (I)

- Three groups of microtubules
  - Kinetochore MTs
  - Interpolar MTs
  - Astral MTs

- Molecular motors play critical roles in maintaining the dynamic architecture of the mitotic spindle.
Prometaphase: Mitotic Spindle Organization (II)

- Constant addition of tubulin at MT plus ends is balanced by depolymerization at MT minus ends. This generates microtubule flux.

Spindle Assembly: MT Organization (I)

- Two pathways of microtubule assembly
  - Centrosome-mediated assembly
  - Centrosome-independent assembly

- Centrosome-independent spindle assembly depends on a Ran-GTP gradient.
Spindle Assembly: Bipolar Attachment (II)

- Two mechanisms to establish bipolar attachment of microtubule and chromosomes
  - Search and capture
  - Chromosome mediated MT growth
Error Correction & Spindle Checkpoint

- Tension between sister chromatids is essential to error detection and correction.

- MAD1/2 & Aurora-B kinase play critical roles in spindle checkpoint.

Metaphase

- Chromosomes become aligned at the metaphase plate.
- Microtubules undergo constant poleward flux.
- Chromosomes oscillation during metaphase.
- APC/C promotes the degradation of securin.
Anaphase A

- Movement of sister chromatids to the poles requires shortening of kinetochore MTs.

- Anaphase A follows activation of APC/C\textsubscript{Cdc20}.

- After spindle checkpoint is turned off, APC/C\textsubscript{Cdc20} triggers the degradation of securin.

- Reduced securin level allows separase to cleave cohesin.
Anaphase B

• Spindle elongation pushes spindle poles apart in Anaphase B.

• Chromosome movement is driven by two factors
  - microtubule shortening and growth
  - microtubule flux

• Spindle elongation
  - Antiparallel sliding of microtubules
  - Microtubule growth
  - Spindle pole motility
Telophase

- Nuclear envelope starts to reassemble in late anaphase and is completed in telophase.

- Ran-GTP mediates nuclear envelope assembly.

- Nuclear lamina reassembles through recycling of disassembled lamin subunits.
Cytokinesis (I)

- Two daughter cells become separated through cytokinesis.
- Formation of the contractile ring requires actin and myosin-II.
- Separation of two daughter cells is accompanied by constriction and disassembly of the contractile ring.
Cytokinesis (II)

- Cytokinesis requires membrane addition and abscission.
- Secretory vesicles from the Golgi provides new membrane.
- Midbody contains many proteins involved in membrane trafficking.
- Intracellular bridges may remain open to connect cells.
Exit From Mitosis

- Cdk1 must be inactivated for exit from mitosis.

- Much of what is known of exit from mitosis comes from budding yeast.

- Exit from mitosis in yeasts is mediated by the MEN GTPase.

- Lowered Cdk activities allow the release of MEN GTPases.

- Released MEN GTPases activate Cdc14p, which inhibits Cdk5.
Questions?