Outline

• Overview of the cell cycle
• Regulatory mechanisms controlling cell cycle
  • Progression of the cell cycle
  • Checkpoint of the cell cycle
• Phases of the cell cycle
  • G1/S transition
  • S phase initiation
  • G2/M transition
  • Anaphase initiation
• DNA damage Checkpoint
• Medications interfering cell cycle
• P53 dependent apoptosis
Phases of the cell cycle

- G0
- G1: Chromosome decondensation, re-formation of nuclear envelope, cytokinesis
- G2: Chromosome condensation, nuclear envelope breakdown, chromosome segregation
- S: DNA synthesis
- M: Daughter cells

Sister chromatids
Phases of the cell cycle and its DNA content

- **G1** (2n)
- **S** (2n → 4n)
- **G2** (4n)
- **M** (4n → 2n)

Cell cycle: G1-S-G2-M
Timing for each phases of the cell cycle

**M-phase**: mitosis and cytokinesis

**Interphase**

**G1**: (Growth phase 1)

**G0**: quiescent or senescent

**S**: DNA replication

**G2**: (Growth phase 2)

Restriction point for cell ready to undergo Mitosis

- **G0 Phase**: Terminally differentiated cells withdraw from cell cycle indefinitely.
- **G0**: Reentry point. A cell returning from G0 enters at early G1 phase.
- **G1 Phase**: RNA and protein synthesis. No DNA synthesis.

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Overview of cell cycle control

• Two irreversible points in cell cycle
  – Replication of genetic material
  – Separation of sister chromatids

• Cell cycle regulation is controlled by Go&Stop signal
  – Progression & Checkpoints
Regulatory mechanism controlling cell cycle

Cyclin-Cyclin dependent kinase (Cdk) system

- **Cdk**: Cyclin-dependent kinase
- **Cyclin**: Cyclin
- **Wee1**: Inhibitory kinase
- **CAK**: CDK-activating kinase
- **CDC25**: Phosphatase

Positive Feedback

- Progression G1/S through Cyclin E/Cdk activity
- Progression G2/M through Cyclin B/Cdk activity
Cyclin-Cdk is controlled by ubiquitin proteasome system during G1/S and G2/M progression
Enzymatic cascade in Ubiquitin proteasome system

G1/S-Cyclin
SCF
APC
M-Cyclin
Regulatory mechanism controlling cell cycle

S / M phase kinase: cdk + cyclin
Regulatory mechanism controlling cell cycle

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Regulation of cell cycle progression

Mitogenic stimuli → Cyclin D → Retinoblastoma protein (RB) → E2F

- E2F activates G1/S-phase cyclin and Cyclin A
- G1/S-phase cyclin promotes DNA replication
- Cyclin A is involved in other transcriptional activity
- Cyclin B participates in cell cycle progression
The cell cycle is also controlled by inhibition.

**G_2 / M checkpoint**
- Replication completed
- DNA integrity

**Spindle checkpoint**
- Chromosomes attached at metaphase plate

**MPF** = Mitosis Promoting Factor
**APC** = Anaphase Promoting Complex

**G_1 / S checkpoint**
- Growth factors

**Cdk/cyclin B (MPF)**

**mitosis**

**cytokinesis**
G1/S transition
Extracellular signals induce cell to commit S-phase entry

**Growth factor**

- Platelet derived growth factor (PDGF) gives mitogenic signal through Receptor tyrosine kinase (RTK)
- made by platelets in blood clots
- binding of PDGF to cell receptors stimulates cell division in connective tissue promoting wound healing
Extracellular signals induce cell to commit S-phase entry

Growth factors, cytokines

MAPK cascade

Phosphorylation of Jun and Fos in nucleus

transcriptional regulation

Cyclins, CDKs

Transcription factor E2F

transcriptional regulation

Enzymes for DNA synthesis

Passage from G1 to S phase

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Growth factor signaling

growth factor

cell surface receptor

protein kinase cascade

cytoplasm

nuclear membrane

nuclear pore

nucleus

cell division

chromosome

E2F

Cdk

Rb

Rb

P

P

P

P
Role of Retinoblastoma sensitivity protein (Rb) in G1/S progression

Mitogenic stimuli → Cyclin D → Retinoblastoma protein (RB) → E2F → G1/S protein expression

Rb dephosphorylated → Rb active

Rb phosphorylated → Rb/E2F

DNA Replication → G1/S-phase cyclin → Cyclin A (inactive) + E2F → Other transcriptional activity → Cyclin B
Expressed Cyclin-Cdk during cell cycle progression

CDK-cyclin combinations

Wee1-like inhibitory kinase
CAK: G1/S-CAK
CDC25

SCF-dependent ubiquitylation of cyclin
Cell cycle Checkpoint (G1/S)

Cell cycle arrest when DNA damage found

p53: transcription factor
Cell cycle Checkpoint protein

p53 phosphorylation
p21: CDK inhibitor

DNA repair & Apoptosis

G1/S checkpoint by p53
Cell cycle Checkpoint (G1/S)

Intact DNA

CDK2
Cyclin E

Active

pRb

E2F
Inactive

transcriptional regulation

Enzymes for DNA synthesis
Passage from G1 to S

pRb

pRb

E2F
Active

Cell division blocked by p53

Cell division occurs normally

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How is p53 activity induced by DNA damage?

DNA damage and cell cycle arrest

**p53**: transcription factor

p53 transcribes p21.
Cell cycle checkpoint

p53 induces E3 MDM2 transcription.

MDM2 ubiquitylates p53.
How is p53 activity induced by DNA damage?

DNA damage and cell cycle arrest

- DNA damage
- DNA kinase

- p53 phosphorylation
- p21: CDK inhibitor

- DNA repair
- Apoptosis

- p53 mutation in >50% of cancers
S phase initiation
(a) Control of S-phase by regulated proteolysis of P27:

(i) P27 is a Cdk inhibitor (CKI) specific for the G1/S-phase Cdk-Cyclin complex

(ii) This proteolysis is initiated by the Cyclin D which phosphorylates P27 making it a target for ubiquitin Conjugating enzyme
Regulation of S phase initiation

(b) Regulation of pre replication complexes:

Cdk's simultaneously

1. Activate initiation of replication
2. Prevent re-initiation at origin sites by phosphorylation & disassociation of McM's from the ORCs (origin recognition complex) which are permanently associated with replication origins.

Minichromosome maintenance (McM) (DNA helicase)
G2/M transition
Regulatory mechanism controlling cell cycle

Cyclin-Cyclin dependent kinase (Cdk) system

Cdk

Cyclin

Cyclin-Cdk

Wee-1

CAK

Cdc25

inactive

inactive

inactive

active

G2 → Mitosis
Initiation of Anaphase
(Spindle assembly assembly checkpoint)
Model for Induction of Anaphase by Regulation of Cohesin Complexes

• “Anaphase inhibitor” (Securin) is involved in the stabilization of a multicompartment of proteins called cohesins

• Cohesins tie together sister chromatids at the centromeric attachments and other sites along the chromatid pairs and maintain this association despite the pulling of the kinetochore attached spindle fibers
Sister chromatid pairing is mediated by the cohesin complex in which Smc1/Smc3 together with Scc1/Scc3 form a bridge between the two chromatids.

Scc1 is the critical protein that serves as a “linker” between cohesin complexes on the two sister chromatids.

APC/Cdc20 mediates release of the anaphase inhibitor (securin) by activating securin degradation resulting in activation of separase and cleavage of Scc1 linkages on the cohesin complexes.
Regulation of Mitotic Exit

- Regulated degradation of mitotic cyclins is a result of a corresponding activation of APC activity via phosphorylation by mitotic cdk and Cdh-1 binding.
- This is an example of two protein complexes which regulate each other in concert with cell cycle regulation.
Summary of the Dual Effect of APC on Mitosis

- Active Separase breaks Cohesin links

- Securin

- $P$ + Cdh-1

- Cdc20

- MPF

- Cyclin B

- Cdc2

- APC

- G2

- Prophase

- Metaphase

- Anaphase

- Telophase

- G1
Summary of the Dual Effect of APC on Mitosis
DNA damage checkpoint throughout the cell cycle
Signal transduction of checkpoint responses

- DNA damage
  - Sensors
  - Mediators
  - Transducers
  - Effectors

- Repair
- Cell cycle arrest
- Apoptosis
DNA damage checkpoint throughout the cell cycle

Sensor

Mediator

Transducer

Effector
Cell cycle arrest in response to DNA damage
DNA damage induces cell cycle arrest

DNA repair and apoptosis
Summary of cell cycle arrest

[Diagram of cell cycle arrest with checkpoints and regulators]
Medications interfering cell cycle

Cancer drugs—cell cycle

Vinca alkaloids and taxols

G2
Synthesis of components needed for mitosis

S
DNA synthesis

Go
Resting

Differentiation

G1
Synthesis of components needed for DNA synthesis

Antimetabolites

Bleomycin

Etoposide

Antineoplastics

Nucleotide synthesis → DNA → RNA → protein → cellular division

Methotrexate, 5-FU:
↓ thymidine synthesis
6-MP:
↓ purine synthesis

Alkylating agents, cisplatin:
cross-link DNA
Dactinomycin, doxorubicin:
DNA intercalators
Etoposide:
inhibits topoisomerase II

Vinca alkaloids:
inhibit microtubule formation

Pacitaxel:
inhibits microtubule disassembly

Topoisomerase II inhibitor

1. DNA binding

2. ATP binding

3. Cleavage

4. Strand passage

5. Religation

6. Product release

ATPase domain

DNA cleavage core domains

Murl, YacG, MfpA, Qnr, simocyclinone D8

Etoposide

Doxorubicin

P, ADP

ADP

ADP

P,
Spindle assembly inhibitor

Paclitaxel

CDK1, Cyclin B

CDK1, Cyclin A

CDK4/6, Cyclin D

CDK2, Cyclin E

p27, Cip/Kip-class CKI

Cell cycle
Bleomycin induces dsDNA cleavage

Diagram showing the mechanism of Bleomycin-induced DNA cleavage.
Bleomycin activates cell cycle arrest, DNA repair, and apoptosis.
Apoptosis

3 Granzyme B pathway
Plasma membrane
Cytosol

1 Extrinsic pathway
FasL/TNFα
Death receptors

2 Intrinsic pathway
FADD
Caspase-8
BID
Caspase-8

BAX-BAK channels
Mitochondrion

BCL-2 subfamily
BAD
HRK
BMF
BIK
NOXA
PUMA
BIM
BH3-only proteins

Cytochrome c

Caspase-9

Caspase-3
Caspase-7
Caspase-3
Caspase-6
Caspase-2
Caspase-8
Caspase-10
P53 dependent apoptosis

- Transcriptional activation; proapoptotic genes (Puma, Noxa and Bax)
- Transcriptional independent process
  - Antagonise Bcl-X<sub>L</sub> and Bcl-2 complex through Bax and Bak oligomerization
  - Disruption of mitochondrial structure; complex formation with cyclophilin D
Why is it important

Between G1 and S phase, cell wish to assure whether cell is ready for cell division

This checkpoint

S is stand for synthesis
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Prophase

Metaphase

Telophase

Anaphase

G2

G1

Mitosis

mm pp

m p

+ m p
Cell cycle and apoptosis

1. DNA pre-replication complexes assemble at origins
2. G₁ cyclin-CDK inactivates Cdh1
3. G₁ cyclin-CDK activates expression of S-phase cyclin CDK components
4. G₁ cyclin-CDK phosphorylates S-phase inhibitor
5. SCF/proteasome degrades phosphorylated S-phase cyclin-CDK inhibitor
6. S-phase cyclin-CDK activates pre-replication complexes
7. Mitotic cyclin-CDK activates early mitotic events
8. APC-Cdc20/proteasome degrades securin
9. APC-Cdh1/proteasome degrades mitotic cyclins

Telophase and cytokinesis

DNA replication

Metaphase

Anaphase

Mitotic cyclin-CDK

G₁ cyclin-CDK

Inhibitor

S-phase cyclin-CDK

SCF

M

G₂

S

Mid-late G₁

Restriction point

Early G₁

G₁ cyclin-CDK

G₁ cyclin-CDK

Inhibitor
Cyclin-Cdk is controlled by ubiquitin proteasome system during G1/S progression

Cyclin-dependent kinase (CDK): key molecule

Cyclin: activator

Wee1: inhibitory kinase

CAK: CDK-activating kinase

CDC25: phosphatase

Mitosis (M-phase) controlled by CDK and cyclin
Role of SCF to regulate G1/S progression
Cyclin-Cdk is controlled by ubiquitin proteasome system during G1/S progression.

**M-phase**

**M-cyclin**

**APC E3 system**

**G1/S-phase**

**G1/S-cyclins**

**SCF E3 system**