Cell Cycle – Control of Cell Proliferation

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1 fertilized egg

In Adults:

100 000 000 000 000 cells

≈4.000.000 cell divisions / sec.
1. Principles of the cell cycle

   Cell Cycle Checkpoints

2. The restriction point

3. CDKs – central cell cycle regulators

4. CDK inhibitors

5. The retinoblastoma protein

6. The RB-E2F pathway
Central Aims of the Cell Cycle

1. Duplication of the genome

2. Separation of the duplicated genetic material (and other cellular compounds) into two daughter cells
Four Cell Cycle Phases

- **Gap-phase 1 (G₁)**
- **DNA Synthesis (S)**
- **Gap-phase 2 (G₂)**
- **Mitosis and Cytokinesis (M)**
Principles of the eucaryotic cell cycle:

1. One and only one replication of the entire genome per cell cycle

2. Initiation of a subsequent cell cycle phase requires proper termination of the preceding phase
$G_0$-phase

Restriction point
Why separate cell cycle phases?

The temporal separation of DNA replikation and mitosis permits the incorporation of control mechanisms in the eucaryotic cell cycle. These control mechanisms are called Checkpoints.
**Checkpoints**

**Checkpoint:** a process within the cell cycle, which controls the transition from one cell cycle state into the next state

- Checkpoints secure e.g. that DNA replication is completed before mitosis can be initiated.

- Checkpoints secure genomic stability by arresting cell cycle progression upon DNA damage.
**Important Checkpoints**

- **G₂/M Checkpoint**
  - ✓ DNA replicated?
  - ✓ DNA damage?

- **Spindel Checkpoint**
  - ✓ all chromosomes attached to the spindle?

- **G₁/S Checkpoint**
  - ✓ size?
  - ✓ DNA damage?

- **M Phase**
  - ✓ mitogens anti-mitogenic signals?
  - ✓ differentiation signals?
Important aims of cell cycle control mechanisms:

1. Restrict cell divisions to precisely the required numbers, e.g. induced divisions after wounding or cell death
   - Avoids hypo- and hyperproliferation

2. Warrant one, and only one DNA replication of all regions of the genome per cell cycle.
   - Avoids genetic instability

3. Precise segregation of the genome into both daughter cells.
   - Avoids genetic instability

4. Prevention of replication or segregation of damaged DNA
   - Avoids the multiplication of damaged DNA
Consequences of misregulated cell cycle control: Genetic instability of tumor cells

Aneuploidy: abnormal number of chromosomes (extra or missing)

Chromosomal translocations: Rearrangements of chromosome parts between nonhomologues chromosomes
Length of cell cycle phases

**Eucaryotes:**
- Early frog embryo cells: 30 min
- Yeast: 1.5 - 3 h
- Cells in the epithelium of the small intestine: 12 h
- Proliferating mammalian cells: 18-24 h
- Fibroblasts in culture: 20 h
- Human liver cells: 1 year

**Duplication of bacteria:**
- E. coli: 20 - 25 min
1. **Principles of the cell cycle**

2. **The restriction point**

3. **CDKs – central cell cycle regulators**

4. **CDK inhibitors**

5. **The retinoblastoma protein**

6. **The RB–E2F pathway**
The Cell Cycle

**Mitosis and cytokinesis**

- **S** - DNA-Synthesis
- **G0** - Phase / quiescence:
  - no cell divisions
  - no DNA-replikation
  - can persist hours, days or years

**Exit from the cell cycle:**
- senescence
- terminal differentiation
- quiescence

**G0-phase / quiescence:**
- no cell divisions
- no DNA-replikation
- can persist hours, days or years

**Restriction Point**
- Mitogens ?
- Antimitogens ?
- Growth factors ?
- Differentiation signals ?


Fig. 1. A, Heterophasic S/02 binucleate cell at t = 0 after fusion. The S nucleus was prelabelled with 3H-thymidine. B, Heterophasic N/02 binucleate cell at t = 6 h after fusion and incubation with 3H-thymidine. The increased intensity of labelling of the S nucleus as compared with that in A arises from continued DNA synthesis after fusion. There was no uptake of 3H-thymidine by the N2 nucleus. C, Homophasic S/02 binucleate cell at t = 6 h after fusion and incubation with 3H-thymidine. The intensity of labelling in each of the nuclei is comparable with that in the S nucleus in B. D, Heterophasic G1/G2 binucleate cell in synchronous mitosis (no colcemid treatment was given). 62 nuclei were prelabelled. Note a slightly less condensed state of the chromosomes of the unlabelled (G1) nucleus.
Fusion of G1, S or G2 cells with mitotic cells leads to nuclear envelope breakdown and DNA condensation in the heterocaryon.

Mitotic cells contain a factor (“MPF” - Mitosis promoting factor) which can induce mitosis in cells of other cell cycle phases.

Fusion of G1 cells with S-phase cells results in heterocaryons, in which the G1 nuclei initiate DNA replikation. S-phase cells only initiate mitosis after completion of DNA replication in G1 cells.

S-phase cells contain a factor which can initiate DNA replication in G1 cells. Checkpoints prevent “premature” entry into mitosis before S-phase is completed in both nuclei.

Heterocaryon experiments

G1, S or G2 + M → M

G1 + S → S

S + G2 → no DNA synthesis in the G2 nucleus, delayed entry into mitosis

G2 nuclei do not re-initiate DNA replication

Existence of a “licensing system”, which labels replicated DNA and prevents re-replication.
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The Nobel Prize in Physiology or Medicine 2001
"for their discoveries of key regulators of the cell cycle"

The Cell Cycle Engine

Leland H. Hartwell
R. Tim Hunt
Sir Paul M. Nurse

Bakers yeast
Sea Urchin
Fission yeast
The Cell Cycle Engine: Cyclin-Dependent Kinases

Cyclin A
(N-term.)

Cdk2

Cdk

Cyclin

Cyclin A
(N-term.)
Cell Cycle Transitions are driven by the oscillating Activity of Cyclin-Dependent Kinases (CDKs)

rel. Cdk-kinase activity

G1-phase kinase  S-phase kinase  M-phase kinase

time

G1  S  G2  M
The Cell Cycle Engine: Cyclin-Dependent Kinases
CDKs are regulated by activating and inhibitory phosphorylations

Assembly factors? spontaneous
CAK KAP
Wee1 / Myt1 Cdc25
Kinases Phosphatases

Cdk

Cyclin

Cdk

Cyclin

Cdk

Cyclin

Cdk

Cyclin

inactive inactive active inactive

CDKs are regulated by activating and inhibitory phosphorylations
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Molecular Brakes: CDK Inhibitor Proteins

G1 arrest
(Lovastatin; TGF-β)

Hengst et al., PNAS 1994
Mechanism of CDK inhibition by p27
Mechanism of CDK inhibition by p27
Cip/Kip Inhibitors are Intrinsically Unstructured Proteins (IUPs)

Lacy et al., *Nature Structural & Molecular Biology*. 2004
The Cip/Kip-Family of CDK Inhibitors

p21
CDK inhibitory domain
164 AA

p27
CDK binding domain
198 AA

p57
Cyclin binding domain
Linker helix
CDK binding domain
316 AA

p21
PCGSK ACRLFGPVD SQQLS RDSDALM AGCIQE ARE RNWFDF VTET PL E-- GDFA MER RGLG LPKLY LPTGP

p27
EHPPK SAPRLFGPVD HEELTRDLEKHC RDMEESQKRW NFDPQNHKL PL E-- GKYE WQEVVEKG SLPEF YRPDR

p57
VLVRT SACRLLFGPVD HEELGRFEL RMRA AEEDAQRMNFQDVP LRGPGRLQ NMEDSE SVPAF YRET-V
Two Families of CDK Inhibitors

Cdk

Cyclin

p27

Cip/Kip family
(Cdk interacting protein; Kinase inhibitory protein)

- p21, p27, p57 (Cip1, Kip1, Kip2)
- bind to a broad spectrum of CDK/cyclins
- bind the CDK/cyclin complex
- conserved N-terminal CDK-inhibitory domain
- may act as activators for cyclin D/Cdk4,6

p16

Ink4 family
(Inhibitor of Cdk4)

- p15, p16, p18, p19 (Ink4 a-d)
- specific for cyclin D / CDK4,6
- bind the CDK subunit
- ankyrin repeat structure
INK4 CDK4,6 Inhibitors

$\text{p16}^{\text{ink4a}}$
What are critical CDK substrates at the G1/S transition?
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The CDK substrat retinoblastom protein (Rb) is a central cell cycle regulator

- Retinoblastoma is a rapidly developing childhood cancer (1/20.000) arising from immature retinal cells
- The Rb protein is a tumor suppressor protein; 45% of the patients carry a heterozygous mutation in Rb1
- Children with (heterogous) mutations in Rb frequently develop tumours in other tissues (Knudsons two-hit hypothesis)
- Viral oncoproteins like E1A and HPV E7 bind Rb and induce cell division in cultured cells
Rb binds E2F and inhibits cell cycle progression

inactive

active
Phosphorylation of Rb releases E2F

inactive

active
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Mitogen induced expression and activation of cyclin D/CDK initiates cell cycle progression.
CDK inhibitors prevent CDK activation
The Rb pathway during the G0/G1/S transition

- G0/G1
- S-Phase Cell
- Antimitogens
- p27
- Cdk4,6
- p16
- Antimitogens
Der Rb Signalweg des G0/G1/S Übergangs
Cancer is a disease of the cell cycle

From: Malumbres and Barbarcid: Nature Reviews Cancer (01). Vol1, 222
Questions

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