DNA replication

Cell division

Xerox machine

Collating machine

DNA

G1

S

M

G2

Chromosome segregation
Alternation of DNA replication and chromosome segregation
The logic of the cell cycle: cell fusion experiments (Rao & Johnson)

S phase cells contain a factor that can trigger DNA replication in G1 cells: S phase Promoting Factor

G1-phase nucleus immediately enters S phase; S-phase nucleus continues DNA replication
The logic of the cell cycle: cell fusion experiments (Rao & Johnson)

block of re-replication

G2-phase nucleus stays in G2; S-phase nucleus continues DNA replication
The Cell Division Cycle

- **mass**: Increases during growth, then decreases before division.

- **DNA nucleus**: Remains constant during growth, then undergoes replication before division.

- **division**: Occurs after DNA replication.
Balanced growth and division

$T_C = \text{cycle time} \quad T_D = \text{mass doubling time}$
Chromosome cycle
- DNA replication
- mitosis
  (precise replication and segregation)

Growth cycle
- cytoplasmic growth
- cell division
  (approx. doubling and halving)
Dissociation of growth and chromosome cycle

**Oogenesis**
- Oocyte grows without dividing (months)
- Egg
- Fertilized egg divides without growing (hours)

**Embryogenesis**
- Fertilized egg divides without growing (hours)
- Adult frog

**Cell Cycles**
- **Standard cell cycle**
  - $G_1$, S, $G_2$, M
  - G1, S, G2, M

- **Early embryonic cell cycle**
  - S, M, S, M, S, M, S
The Nobel Prize in Physiology or Medicine 2001

"for their discoveries of key regulators of the cell cycle"

Leland H. Hartwell
USA
Fred Hutchinson Cancer Research Center Seattle, WA, USA
1939 -

R. Timothy (Tim) Hunt
Great Britain
Imperial Cancer Research Fund London, Great Britain
1943 -

Paul M. Nurse
Great Britain
Imperial Cancer Research Fund London, Great Britain
1949 -
Saccharomyces cerevisiae

Lee Hartwell
Isolation of temperature sensitive $\textit{cdc}^{ts}$ mutants

(a) Identification of $\textit{cdc}$ mutants

- Incubate replica plates of mutagenized cells at 25 °C and 35 °C

- Replate $\textit{ts}$ mutants; incubate at 35 °C until they stop replicating; observe in light microscope

Missing colony at 35 °C

- Non-$\textit{cdc}$ mutant cells

Colony of $\textit{ts}$ mutants

- $\textit{cdc}28^{ts}$ cells
random population of cells before temperature is raised
Phenotype of cdc mutants

asynchronous culture

cdc mutant with problem of mitotic exit
Cdc28 is responsible for the first genetically controlled event.

Fig. 3. The circuitry of the yeast cell cycle. Events connected by an arrow are proposed to be related such that the distal event is dependent for its occurrence upon the prior completion of the proximal event. The abbreviations are the same as in Fig. 1. Numbers refer to cdc genes that are required for progress from one event to the next; HU and TR refer to the DNA synthesis inhibitors hydroxyurea and thymidine, respectively; MF refers to the mating factor, α factor.
Cloning of \( \textit{cdc}^{ts} \) genes

\( \textit{cdc}^{28} \) cells grown at 25 °C

Transform with plasmid library of wild-type \( S. \textit{cerevisiae} \) DNA

Transformed \( \textit{cdc}^{28} \) cells grown at 35 °C

Gene \( X \)

Gene \( Y \)

\( \textit{cdc}^{28} \)

No colony formation

Cells in colony at various cell-cycle stages

Isolate plasmid

\( \textit{cdc}^{28} \)

\textit{Cdc28} is a protein-kinase
Schizosaccharomyces pombe

Sir Paul Nurse

Murdoch Mitchison
wildtype

$cdc2^{ts}$
$cdc25^{ts}$
$cdc13^{ts}$
$wee1^{ts}$

$wee2^{ts} = cdc2^D$

$cdc2^{ts}$

$cdc25^{ts}$

$cdc13^{ts}$
cdc2 is required at two points during the cell cycle

FISSION YEAST (*Schizosaccharomyces pombe*)

cdc2

\[ cdc2^{S.c.} \text{ and } cdc2^{S.p.} \text{ are functional homologs} \]
Do human cells have the same gene?
Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*

Melanie G. Lee & Paul Nurse

Cell Cycle Control Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, UK
cdc25\(^+\) Functions as an Inducer in the Mitotic Control of Fission Yeast

Paul Russell and Paul Nurse
Cell Cycle Control Laboratory
Imperial Cancer Research Fund
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Negative Regulation of Mitosis by wee1\(^+\), a Gene Encoding a Protein Kinase Homolog

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Cell Cycle Control Laboratory
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London WC2A 3PX, England
Yoshio Masui, Ph.D., FRS
Professor Emeritus of Zoology
University of Toronto
Ontario, Canada
Periodic proteins (cyclins) during early embryonic development
Purified Maturation-Promoting Factor Contains the Product of a Xenopus Homolog of the Fission Yeast Cell Cycle Control Gene $c\text{dc}2^+$

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Manfred Lohke,* ‡ Paul Nurse,*
and James Maller*†
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Oxford OX13QU, England
MPF is a cyclin-dependent protein-kinase

Cdk1

CycB
Cyclin and MPF levels during early mitotic cycles

Questions:

What causes the fast disappearance of cyclins?
What is the reason of the delay between cyclin and MPF?
Cycling cell free extract

- Cytoplasm from activated frog eggs
- Nuclei from frog sperm

Cell-free cell cycle: 40–60 min
Anaphase Promoting Complex or Cyclosome

Kim Nasmyth
Anaphase Promoting Complex or Cyclosome

Cdk1 + CycB degradation

Cdk1 + degraded CycB
The G2/M transition is regulated by post-translational modification.

Wee1 is a tyrosine-kinase.
Cdc25 is a tyrosine-phosphatase.
Start of DNA replication is also controlled by proteolysis

G1

inactive Cdk/cyclin complex → phosphorylated CKI → multiubiquitination CKI → degraded CKI → active Cdk/cyclin complex
Regulation of CDK activity

Cdk → Cyclin

Cdk → Cdk → Cyclin

P → Cdk → Cyclin

Cdk → CKI

CKI → CKI → Cdk → Cyclin

CKI → CKI