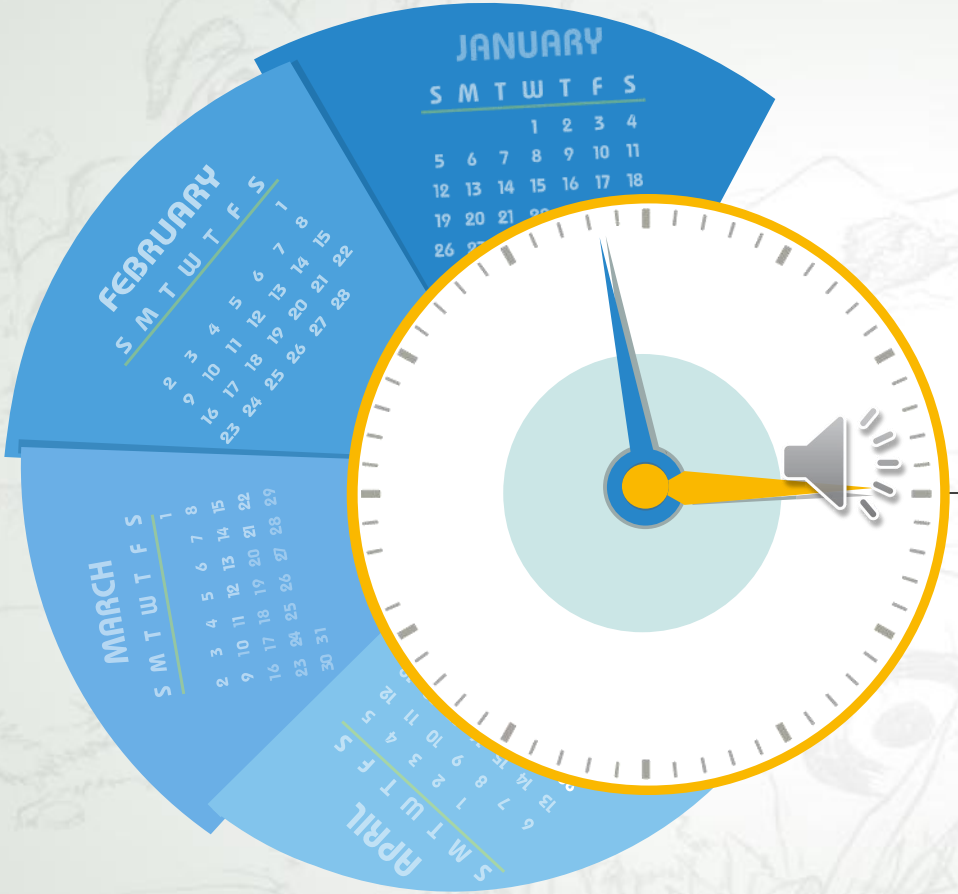
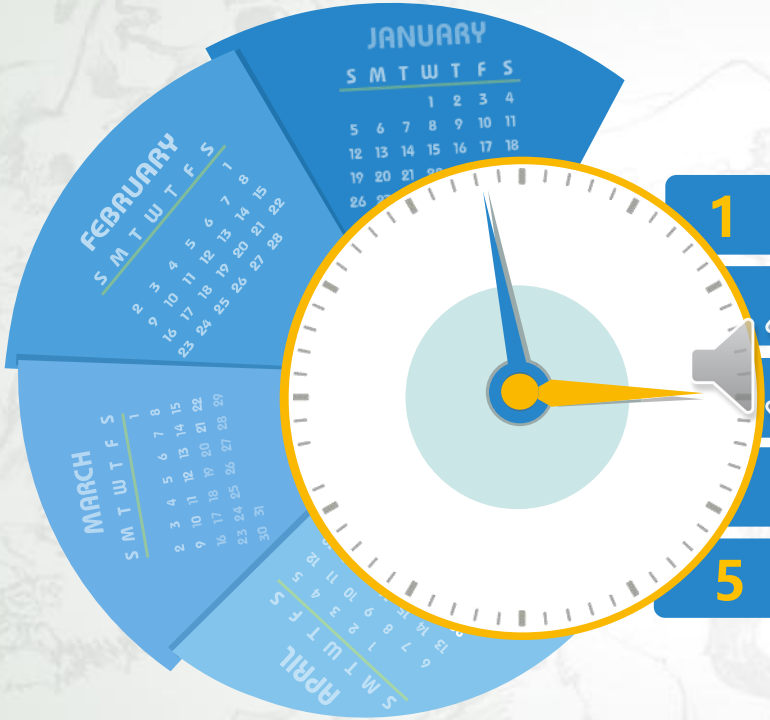


Cell Cycle Regulation

Creative Bioarray

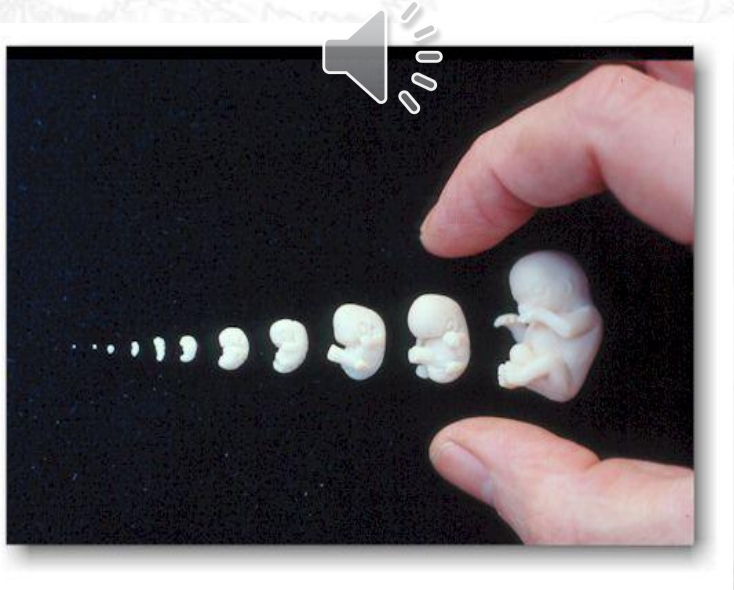
Content



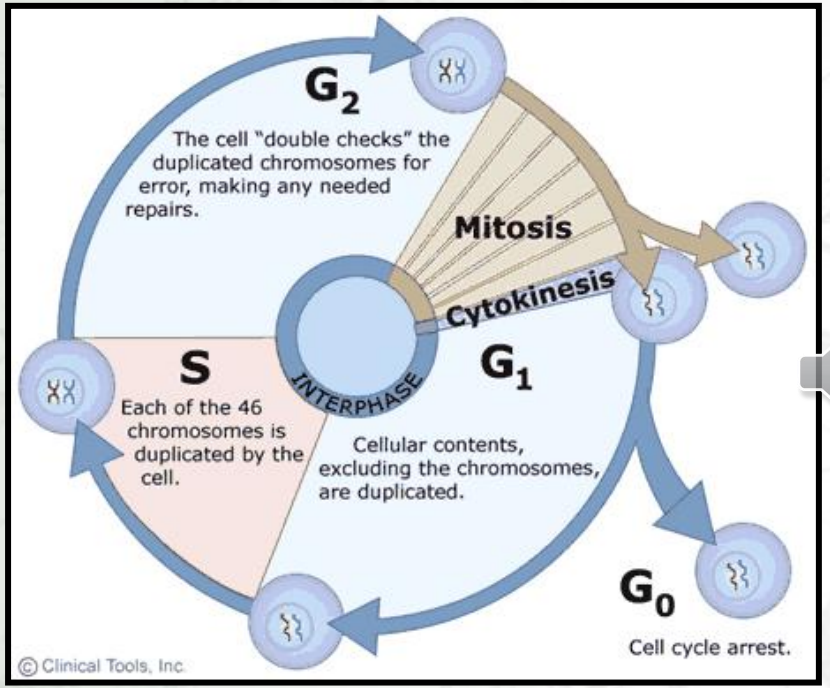
Overview

Cell cycle：DNA replication and cell division

- **Interphase → Mitotic phase → Cytokinesis**



Overview-Phase



Gap 1 (first gap phase)

↓

S phase (synthetic phase)

↓

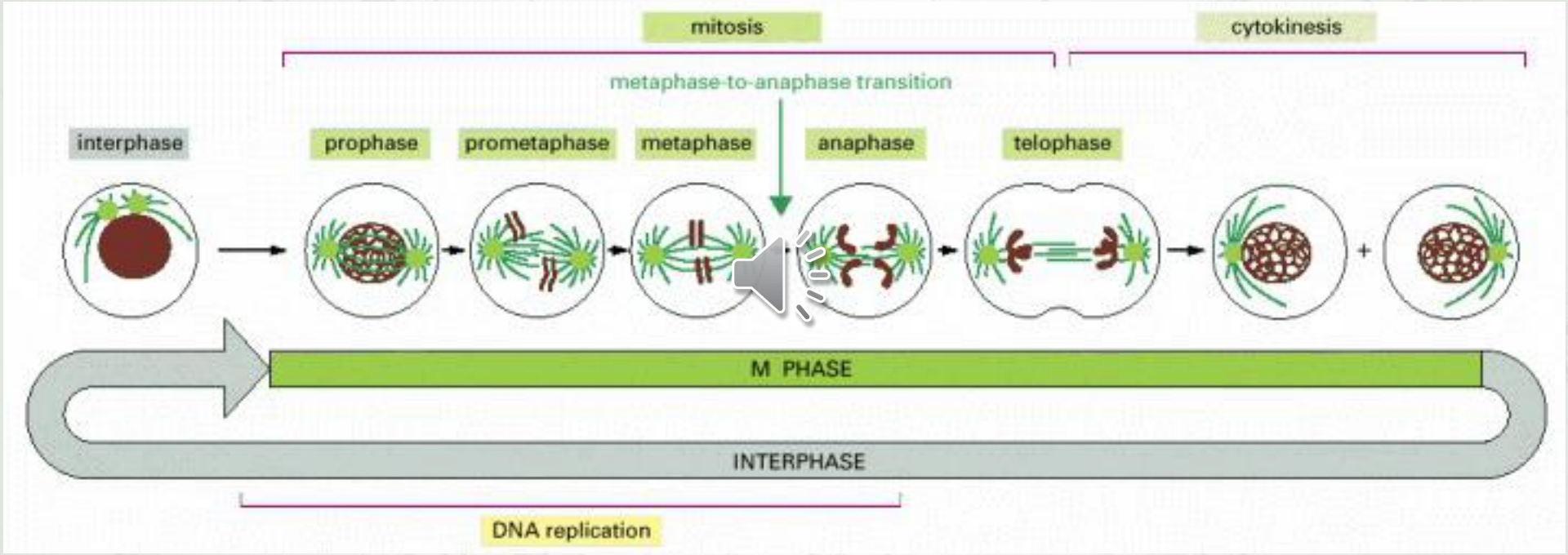
Gap 2 (second gap phase)

↓

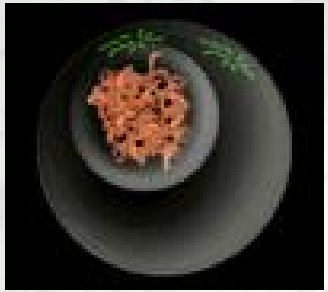
M phase (mitotic phase)

The eukaryotic cell cycle is traditionally divided into four sequential phases: G_1, S, G_2, and M. G_1, S, and G_2 together are called interphase that might occupy 23 hours of a 24 hours cycle, with 1 hour for M phase.

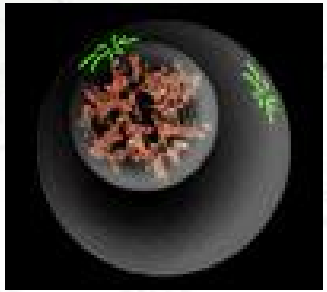
Overview-Phase



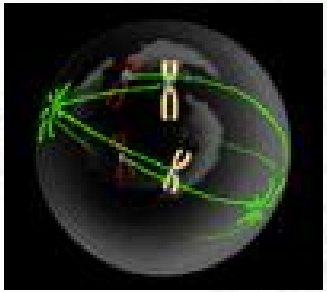
Overview-mitosis



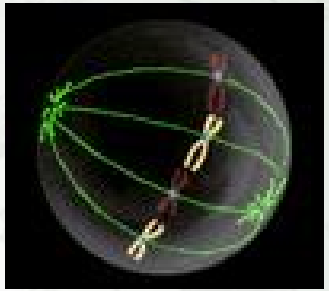
Interphase



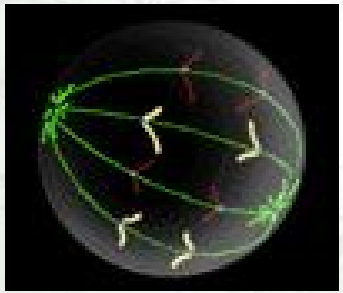
Prophase



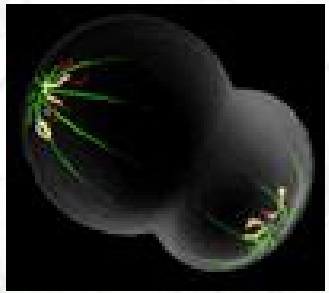
Prometaphase



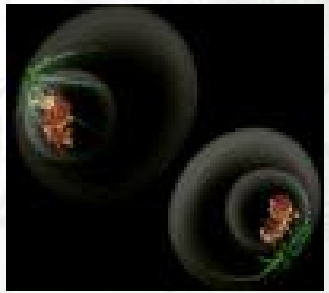
Metaphase



Anaphase



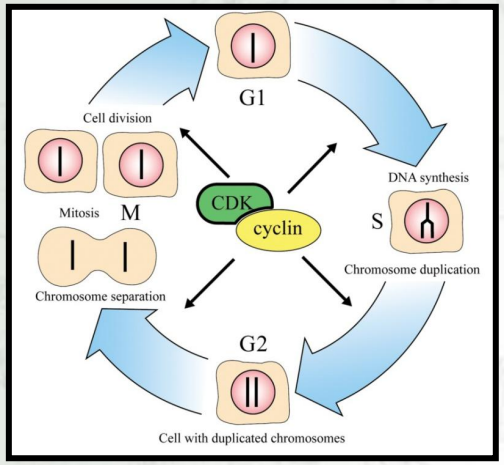
Telophase



Cytokinesis

Classification of Human Cells

- Cycling cells: Epidermal cells, Bone marrow stem cells, Germ cells;
- G0 cells: Hepatocytes, Renal cells;
- Terminally differentiated cells: Nerve cells, Cardiomyocytes.



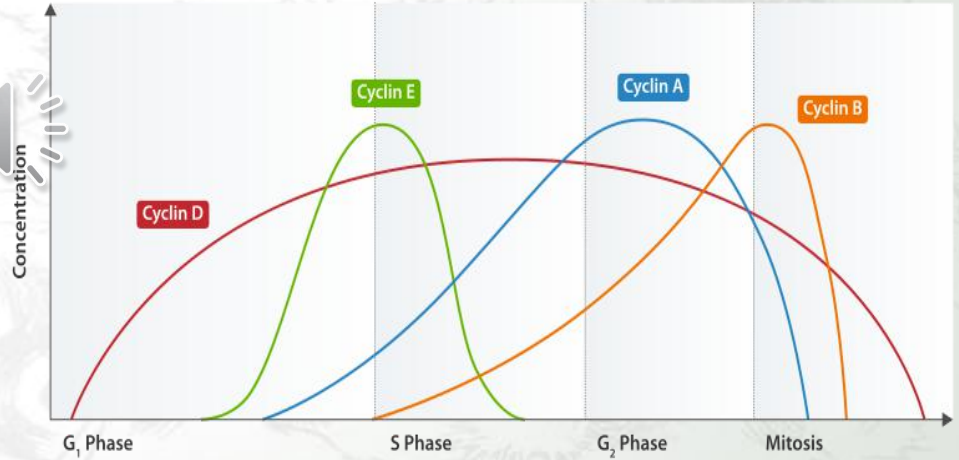
Cell Cycle Regulator

- Cyclin
- Cycle-dependent kinase
- Phosphorylation of CDK
- Cyclin-dependent kinase inhibitor

Cell Cycle Regulator

Cyclin

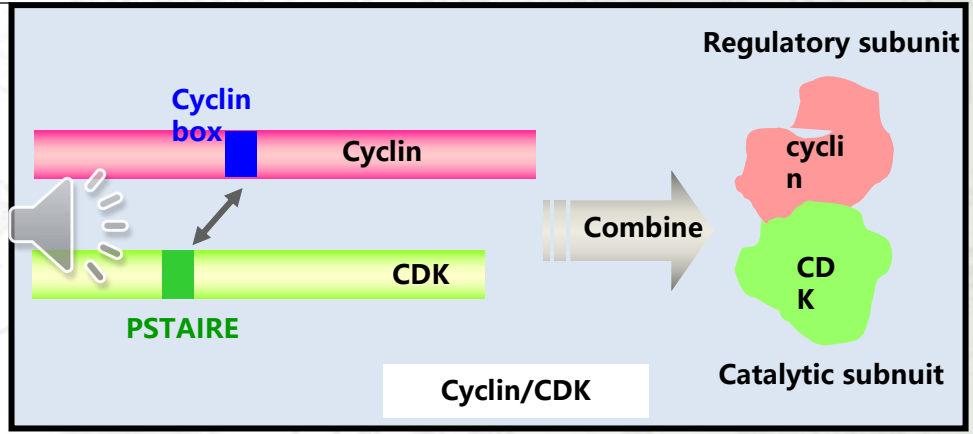
Cyclins are named such because they undergo a constant cycle of synthesis and degradation during cell division. When cyclins are synthesized, they act as an activating protein and bind to CDKs forming a cyclin-CDK complex. This complex then acts as a signal to the cell to pass to the next cell cycle phase. There are two classes of cyclins: mitotic cyclins and G1 cyclins.



Cell Cycle Regulator

Cycle-dependent kinase (CDK)

CDKs are relatively small serine/threonine protein kinases with molecular weights ranging from 34 to 40 KDs. By definition, a CDK binds a regulatory protein called a cyclin for their activation. Without cyclin, CDK has little kinase activity, only the cyclin-CDK complex is an active kinase. Activation and inactivation of these kinases trigger the transition of the cell cycle to subsequent stages.

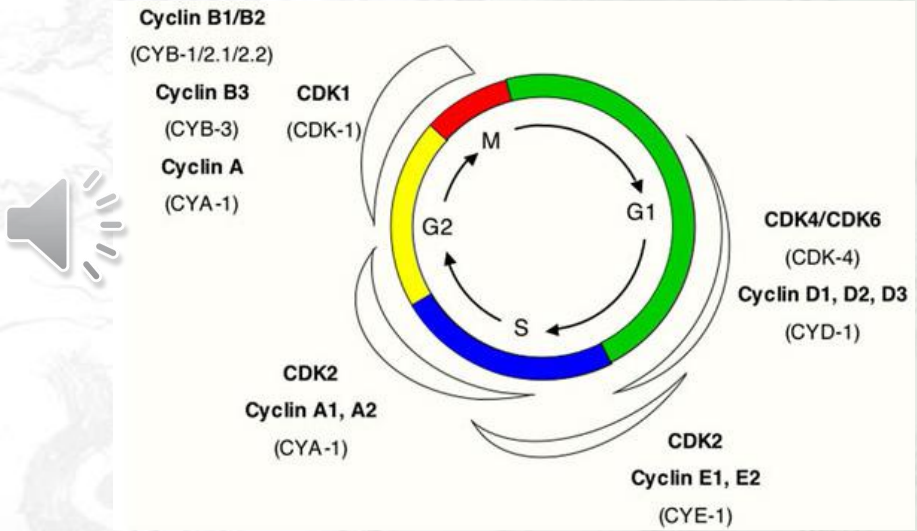


A list of CDKs with their regulation protein and cyclin												
CDK1	CDK2	CDK3	CDK4	CDK5	CDK6	CDK7	CDK8	CDK9	CDK10	CDK11	CDK12	CDK13
Cyclin A, B	Cyclin A, E	Cyclin C	Cyclin D1, D2, D3	CDK 5R1, 5R2	Cyclin D1, D2, D3	Cyclin H	Cyclin C	CyclinT1, T2a, T2b, K	-	Cyclin L	Cyclin L	Cyclin L

Cell Cycle Regulator

Cyclin-CDK complex

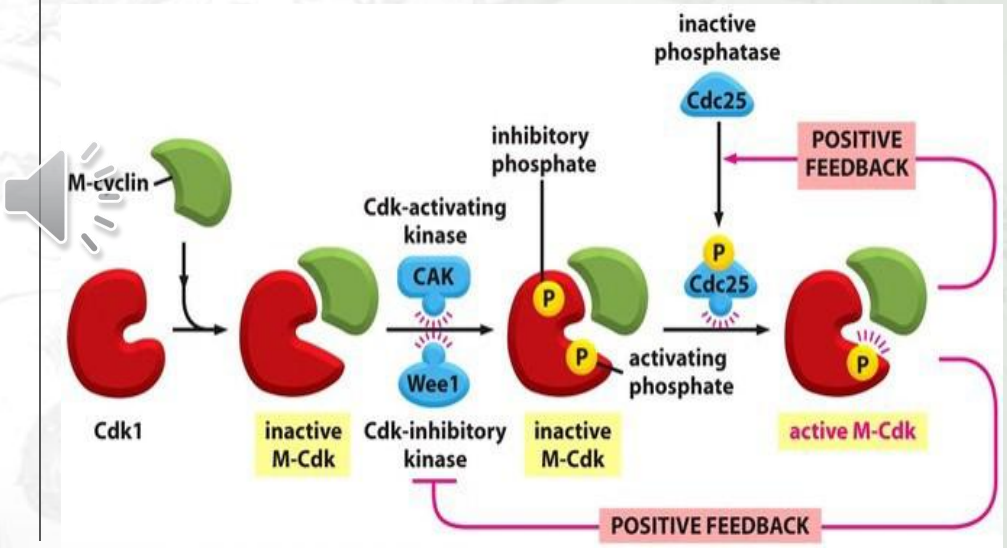
Phase	Cyclin	CDK
G0 phase	Cyclin C	CDK 3
G1 phase	Cyclin D, E	CDK 2, 4, 6
S phase	Cyclin A, E	CDK 2
G2 phase	Cyclin A	CDK 1, 2
M phase	Cyclin B	CDK 1



Phosphorylation of CDK

Full kinase activity requires an activating phosphorylation on a threonine adjacent to the active site. The identity of the CDK-activating kinase (CAK) that performs this phosphorylation.

CDK inhibitory phosphorylation is vital for regulation of the cell cycle. One of the kinases that place the tyrosine phosphate is Wee1, a kinase conserved in all eukaryotes. Phosphatases from the Cdc25 family dephosphorylate both the threonine and the tyrosine.



Cyclin dependent kinase inhibitor, (CDI /CKI)

- **Inhibitors of kinase 4 (INK4) family**

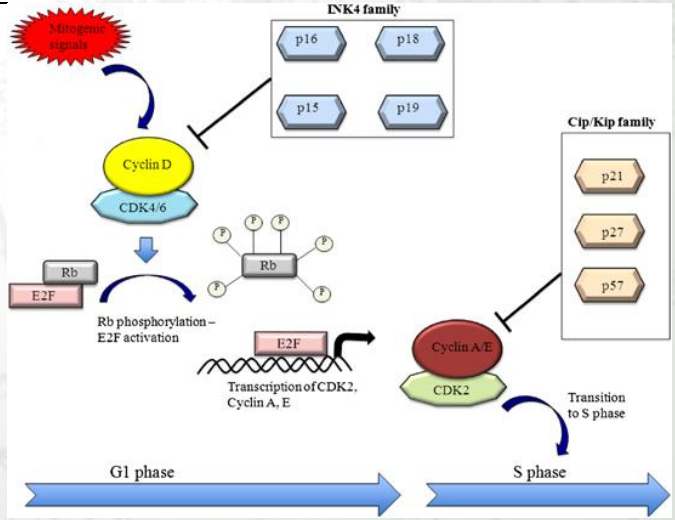
  Members: p16INK4A, p15INK4B,
  p18INK4C, p19INK4D
  Mainly inhibit CDK4、CDK6

- **CDK inhibitory protein (CIP) / Kinase inhibitory protein (KIP) family**

  Members: p21Cip1, p27Kip1, and p57Kip2
  Mainly inhibit CDK2

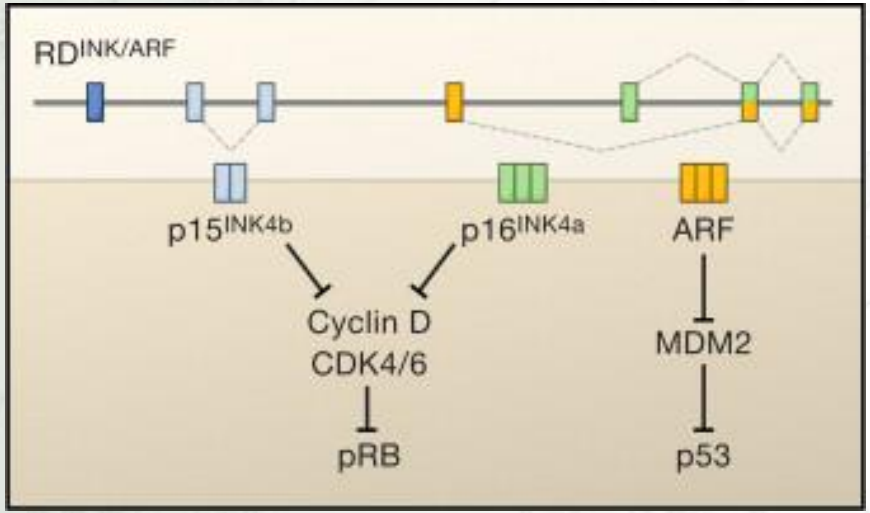
- **Rb/E2F transcriptional regulation protein family**

  Members: p107, p110, and p130
  Mainly inhibit CDK2



Cell Cycle Regulator

Cyclin dependent kinase inhibitor (CDI/CKI)

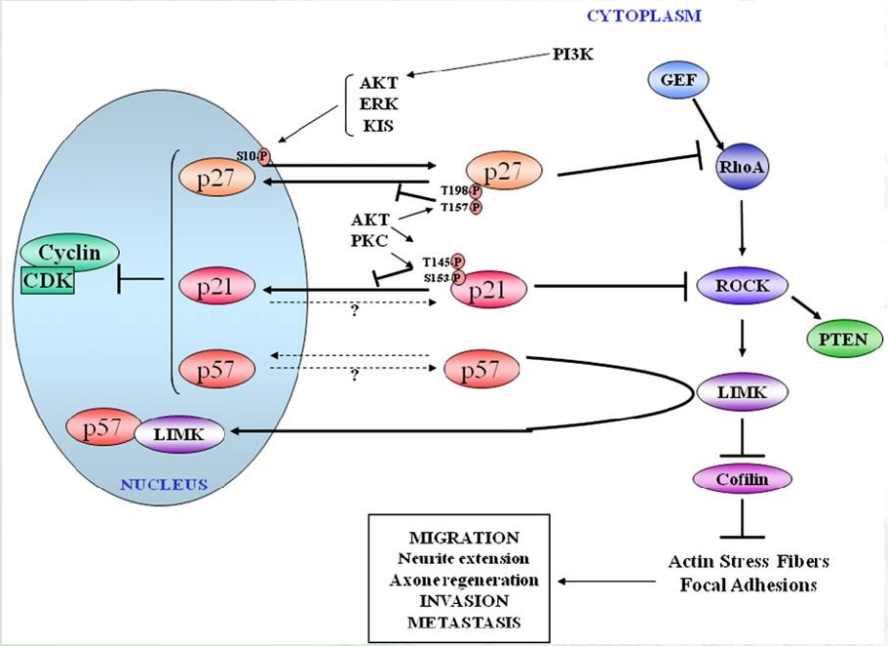


1 INK4 family

Both p15 (CDKN2B gene) and p16 (CDKN2A gene) are members of the INK4 family, which control the mid G1 phase by binding to CDK4 and CDK6, leading to a decreased phosphorylation of target proteins. Overexpression of p16 arrests the cell cycle by inhibiting CDK4/Cyclin D during early G1.

Cell Cycle Regulator

Cyclin dependent kinase inhibitor (CDI/CKI)

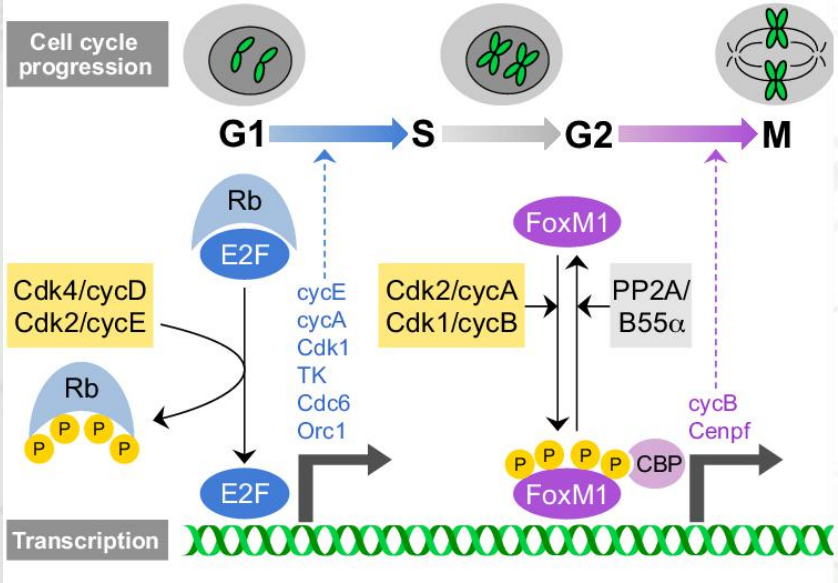


2 CIP/KIP family

CDKs are also regulated by CDK inhibitors p27 (CDKN1B gene), p21 (CDKN 1A gene) and p57 (CDKN 1C gene), which bind to and inhibit both of the G1 CDKs (CDK4 & CDK6). p27 does this by physically blocking the cyclin/CDK complex's interaction with its targets.

Cell Cycle Regulator

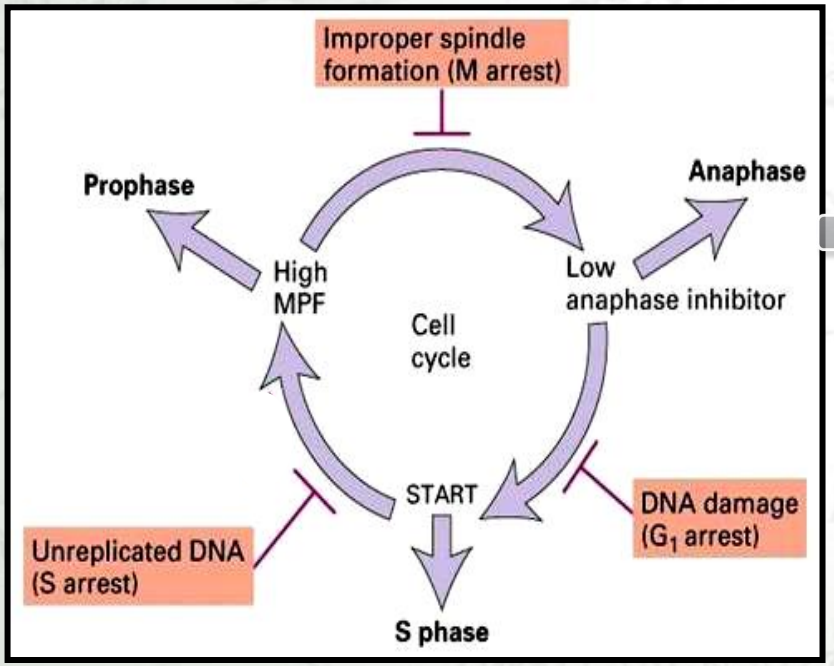
Cyclin dependent kinase inhibitor (CDI/CKI)



3 Rb/E2F family

Another inhibitor called Rb prevents entry into the S phase by binding to E2F transcription factors. E2Fs are transcriptional activators when they act alone but repressors when bound to Rb. The mid-G1 cyclin/CDK complexes partially phosphorylate Rb, reducing its binding to E2Fs; the late G1 complex of CDK2/cyclin E completely phosphorylates it, preventing its binding to E2F. E2Fs can then act as transcriptional activators for genes needed in the S phase.

Checkpoint Mechanism



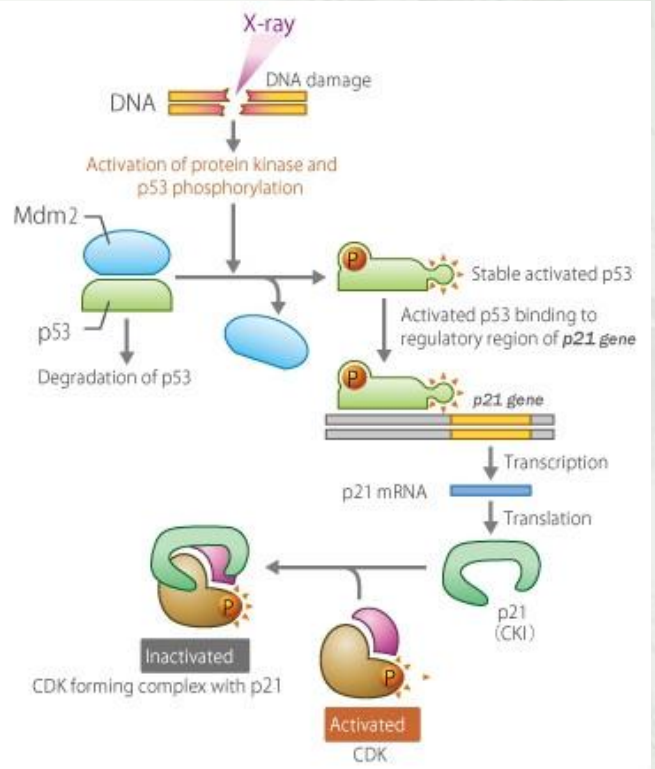
Checkpoint mechanism in cell cycle

- DNA damage checkpoint—G1/S
- DNA replication checkpoint—S/G2
- Spindle assembly checkpoint—G2/M

Checkpoint Mechanism

- DNA damage checkpoint

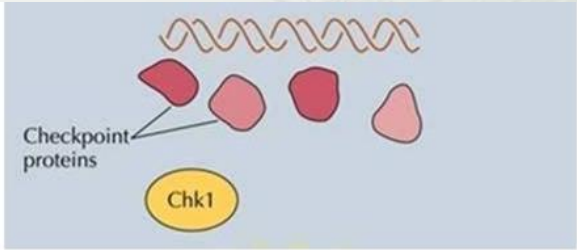
If the DNA is found to be damaged, ATM/R phosphorylate and activate the p 53 protein during the G1, S, and G2 phases.
One role of the p53 protein is to activate the genes of the p21 protein, a type of CKI, to create large amounts of p21 protein, and to inhibitthe actions of cyclin-CDK. As a result, cells cannot move onto the next step until the DNA damage is repaired.

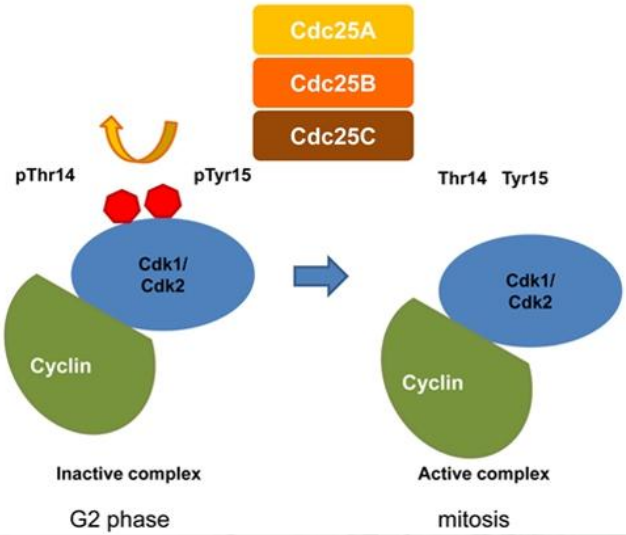


Checkpoint Mechanism

- DNA replication checkpoint

This checkpoint allows the cell cycle to transition to nuclear division after DNA has been adequately replicated. If any part of the DNA has not been replicated, the checkpoint identifies such parts and inhibits the activity of the G2-phase cyclin-CDK, the protein that instructs entry into the M phase.

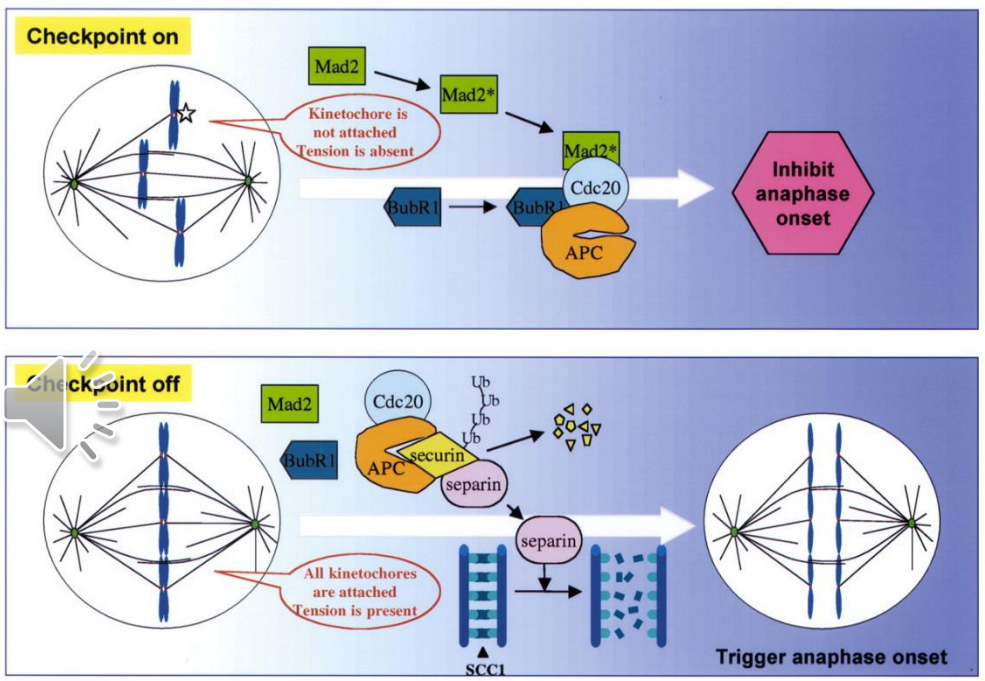




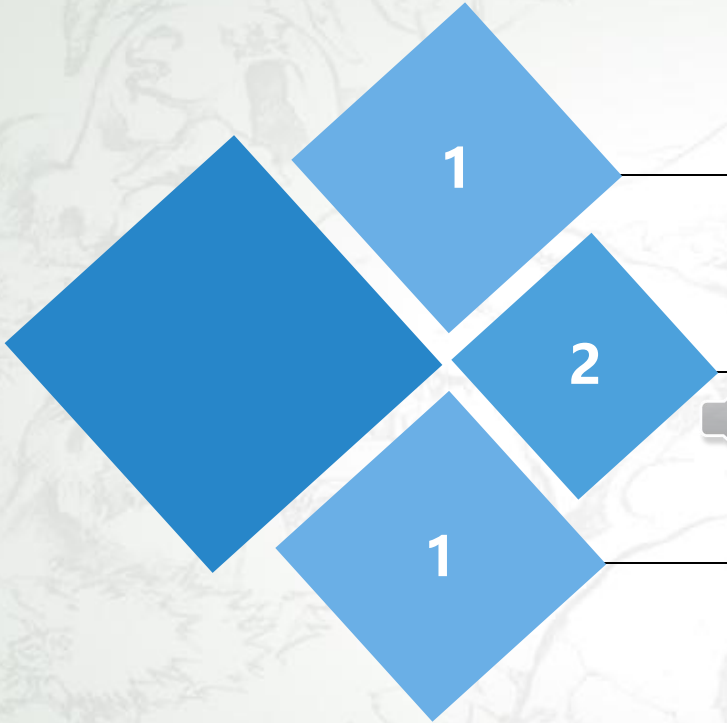
Checkpoint Mechanism

- Spindle assembly checkpoint

Incorrect spindle assembly prevents the adv
ancement into anaphase. The mechanism r
esponsible for ensuringappropriate spindle
assembly is called the spindle assembly che
ckpoint. It has
been recently understood that
the protein Mad2 is involved in this process.



Cell Cycle Dysregulation and Cancer



Cancer cell cycle regulator

Cell cycle regulators and cancer

CDK inhibitors and cancer

Cell-cycle dysregulation is a hallmark of tumor cells. The ability of normal cells to undergo cell-cycle arrest after damage to DNA is crucial for the maintenance of genomic integrity.

Cell Cycle Dysregulation and Cancer

Cell cycle pathways of tumor suppression

- PRb——a key regulator of progression
- P53——the guardian of the genome

Human p53 mutation hotspot and frequency					
Cancer	Frequency	Mutation hotspot	Cancer	Frequency	Mutation hotspot
Lung cancer	56	157, 248, 273	Liver cancer	45	249
Colon cancer	50	175, 245, 248, 273	Glioma	25	175, 248
Ovarian cancer	44	273	Breast cancer	22	175, 248, 273
Gastric cancer	41	Uncertain	leukemia	12	175, 248
Bladder cancer	34	280	Soft tissue sarcoma	31	Uncertain

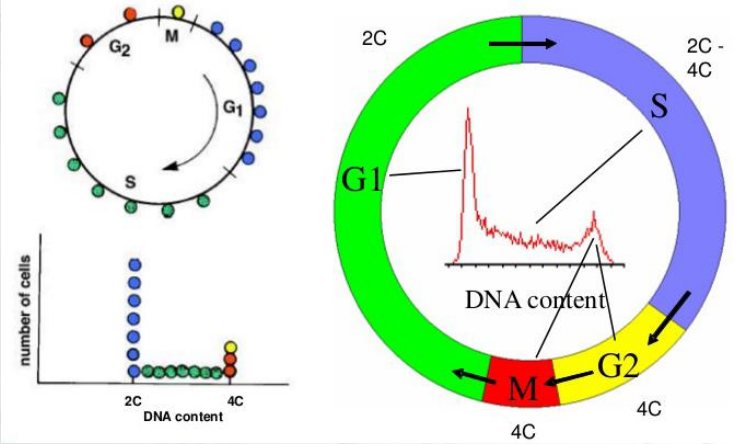
Cell Cycle Dysregulation and Cancer

Cell cycle regulators and cancer			
Protein	Chromosome	Function	Relevance in human cancer
Cyclin A	4 (q25-q31)	Complexed with CDK2 and 1 to regulate S phase and G2-M	Overexpressed in some breast cancer, hepatocellular cancer
Cyclin B1	5 (q13-qter)	Complexed with CDK1 to regulate G2-M	Overexpressed in some breast cancer
Cyclin D1	11q13	Complexed with CDK4/6 to regulate early G1	Overexpressed in multiple tumor
Cyclin D2	12q13	Complexed with CDK4/6 (some cell types) to regulate early G1	Overexpressed in some colorectal tumor
Cyclin E	19q12	Complexed with CDK2 to regulate late G1 and the G1-S transition	Overexpressed in multiple tumors
CDK 1	10	Complexed with cyclin B1 to regulate G2-M	Overexpressed in some breast cancer
CDK 4	12q13	Complexed with D-type cyclins to regulate early G1	Amplified in brain tumors, infrequently mutated in melanomas

Cell Cycle Dysregulation and Cancer

Cyclin-dependent kinase (CDK) inhibitors and cancer			
Protein	Chromosome	Function	Role in human cancer
p21CIP1	6p21	Inhibits multiple CDK/cyclin complexes and PCNA to block G1 and S phase, induced by p53	Rare mutations in prostate, bladder and breast cancer
p27KIP1	12p13	Inhibits multiple CDK/cyclin complexes to induce G1 arrest	Loss of heterozygosity not uncommon; variable loss of protein expression in many malignancies
p57KIP2	11p15.5	Inhibits multiple CDK/cyclin complexes to induce G1 arrest	Few inactivations identified; mutations found in patients with Beckwith-Wiedemann syndrome
p16INK4a	9p21	Inhibits CDK4/6 to induce G1 arrest	Frequently inactivated in cancers, especially melanoma, pancreatic, lung, and bladder cancer
p14ARF	9p21	Blocks MDM2 inhibition of p53, thereby inducing G1 and G2 arrest	Few exclusive deletions identified in melanoma cell lines, gliomas; targeted in acute T-cell leukemia

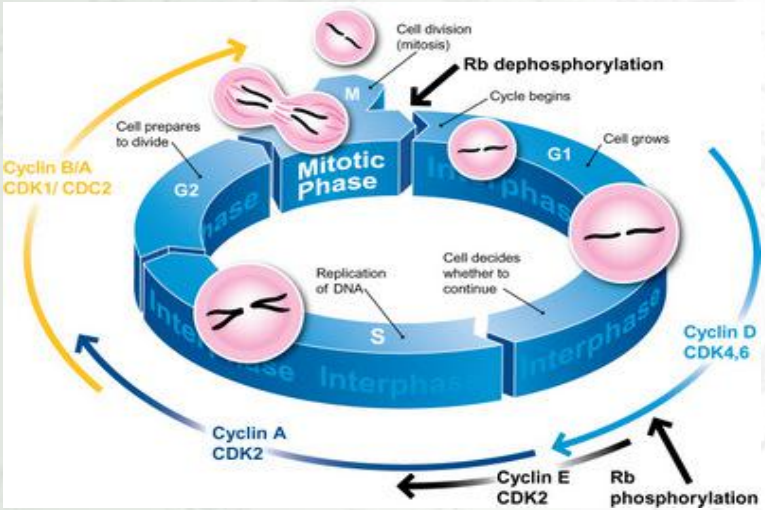
Cell Cycle Assays



DNA Content Analysis

DNA content can be measured by fluorescent staining which allows the cells to differentiate in G0/G1, S phase, and G2/M, as well as exhibits an emission signal proportional to the amount of DNA. DNA content analysis is typically performed on fixed or permeabilized cells using cell permeable nucleic acid dyes or impermeable nucleic acid stains. The most common DNA binding dyes currently in use are the blue-excited propidium iodide (PI) and the UV-excited diamidino-phenylindole (DAPI) dye.

Cell Cycle Assays

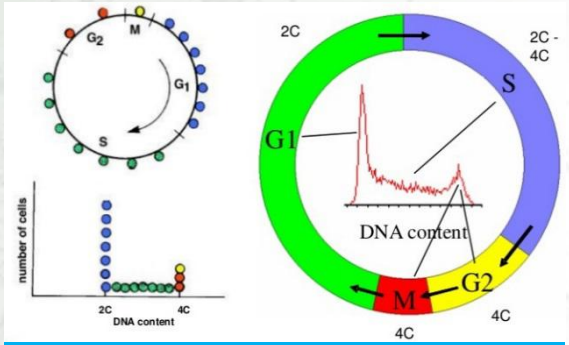


Bivariate Cell Cycle Assay (Cyclins/PI)

Bivariate analysis of DNA content and cyclins, allowing differentiation between G0 and G1 cells, identification of mitotic cells, and measurement relate expression of other intracellular proteins to the cell cycle position.

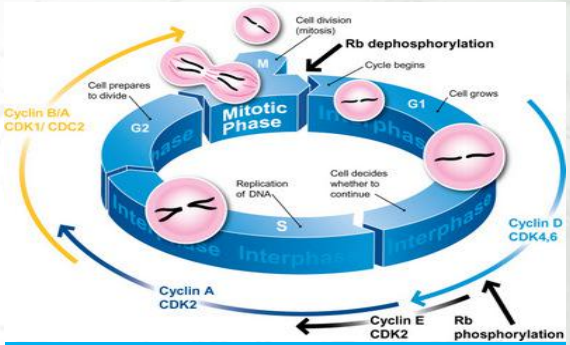
The cell cycle assays are applicable to many areas of life science research and drug development, including cancer biology, apoptosis analysis, drug screening, and measuring health status of cell cultures in bioreactors.

Comparison of Two Methods



Univariate

VS



Bivariate

Classic method to use

Simple operation

Do not provide information on cell cycle kinetics

Allowing differentiation between G0 and G1

Identification of mitotic cells

Complex operation

Time-consuming

Expensive

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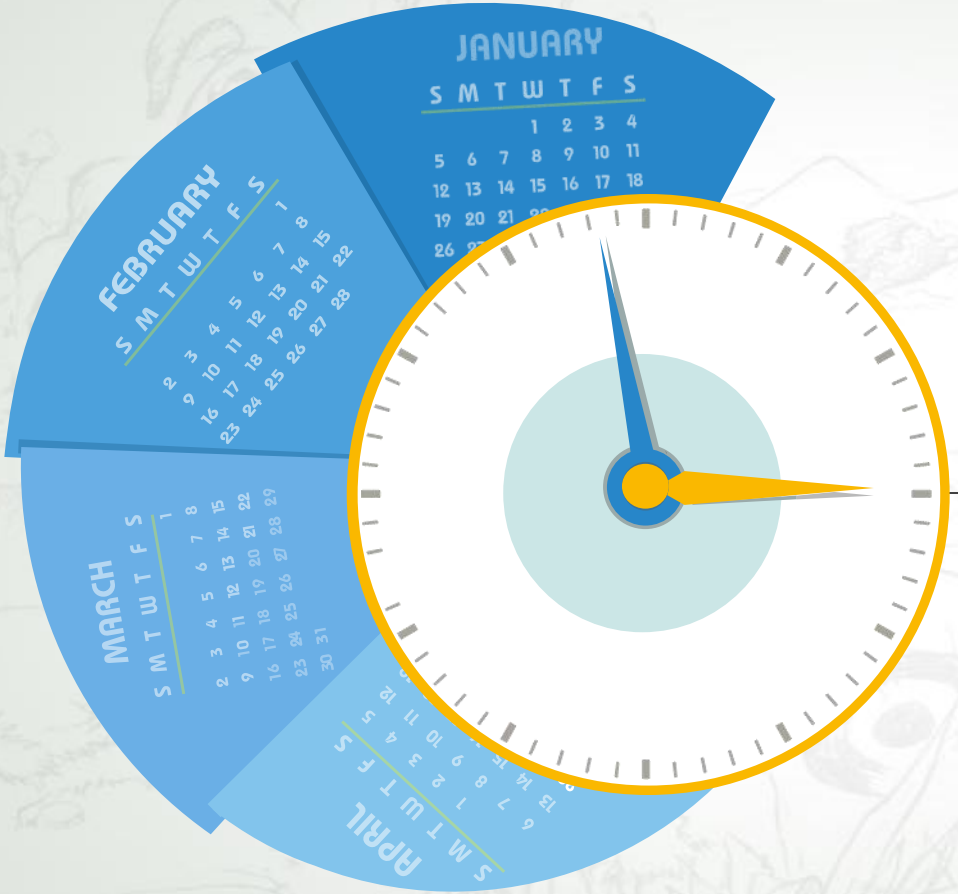

THANK YOU

Creative Bioarray