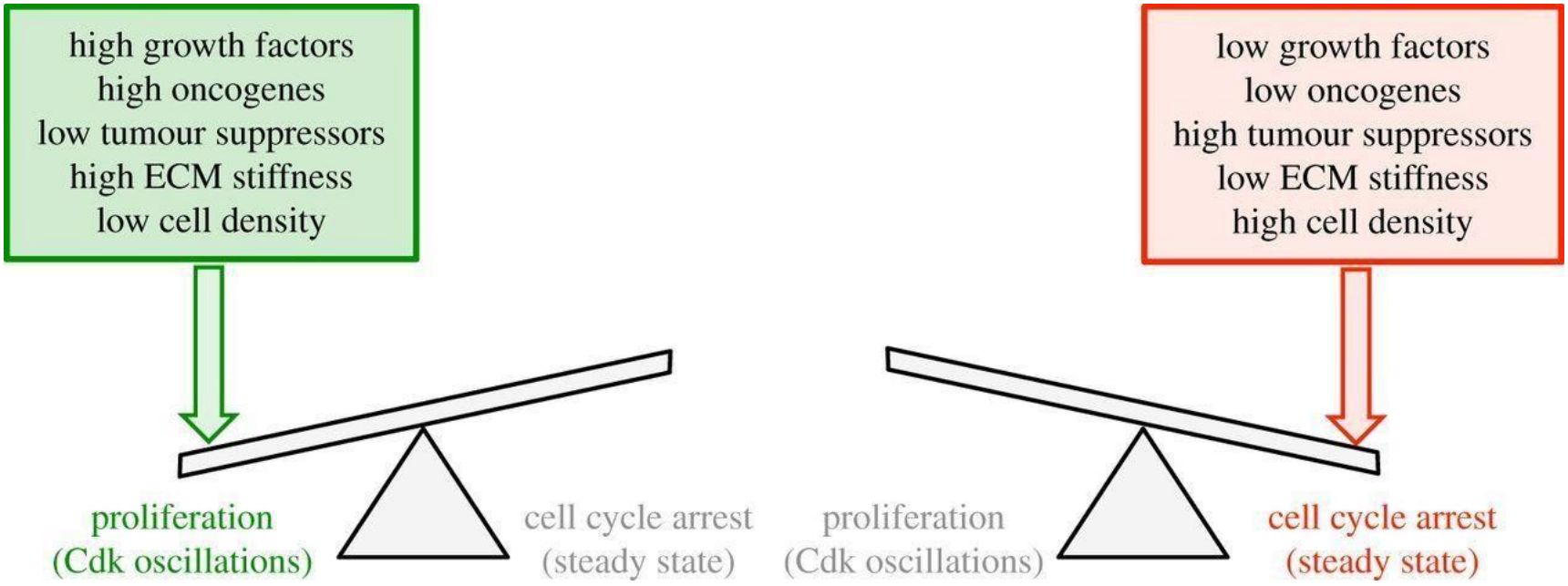
The cell cycle

Lecture 3

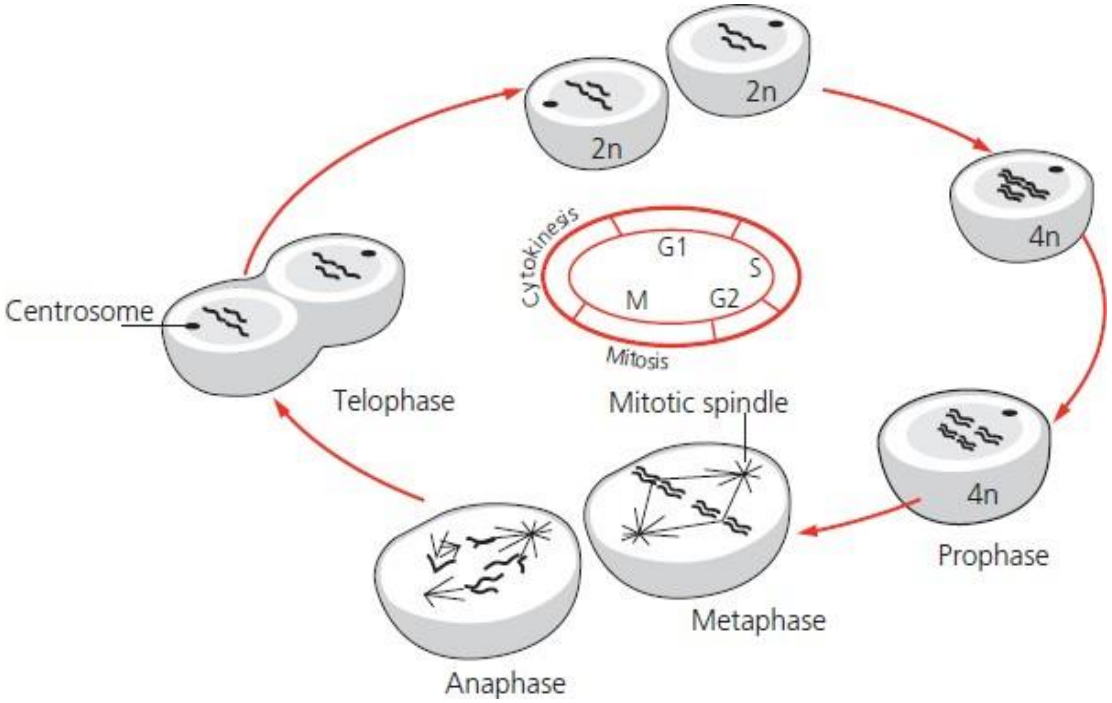
The Balance between cell cycle arrest and cell proliferation



Cell Cycle

- The cell cycle is a series of temporally ordered events that leads a cell to divide itself into two daughter cells.

- Cancer is characterized by **abnormal cell proliferation**.



Cell Cycle Characteristics

- Temporally ordered events.
- It cannot go backwards (Irreversible).

- **Positive** and **negative** feedback loops regulate the function of the molecules involved and ensure the coupling of the following phase on the accomplishment of the previous
- Checkpoints maintain the order of the events in case something goes wrong

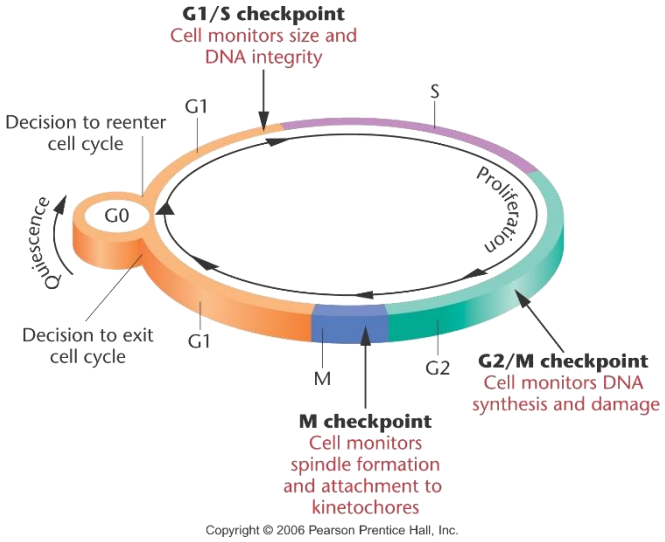
When do cell numbers change ?

- Growth and development (expand pool of cells)
- Tissue turnover (replace lost cells e.g. blood loss)
- Response to injury (add new cells to wound, replace lost cells)
- Physiological changes (e.g. lactation, infection, hypoxia, exercise)
- Need to balance production with loss for tissue homeostasis

Cell Cycle Checkpoints

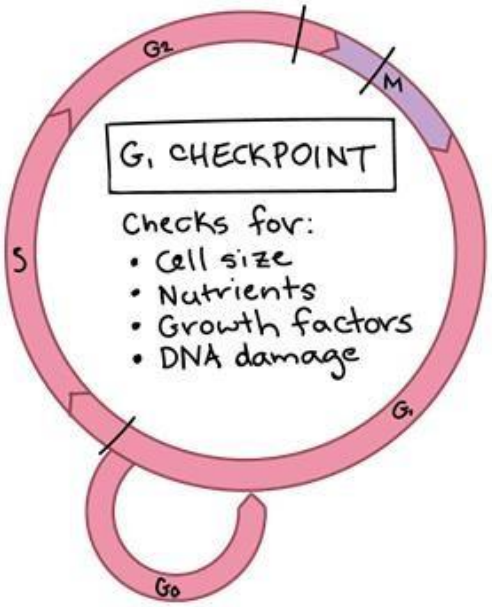
Cell cycle checkpoints a series of biochemical signaling pathways that sense and induce a cellular response to DNA damage, are important for maintaining the integrity of the genome

- Disruption of checkpoint function leads to **genomic and chromosomal instability** leading to mutations that can induce carcinogenesis.

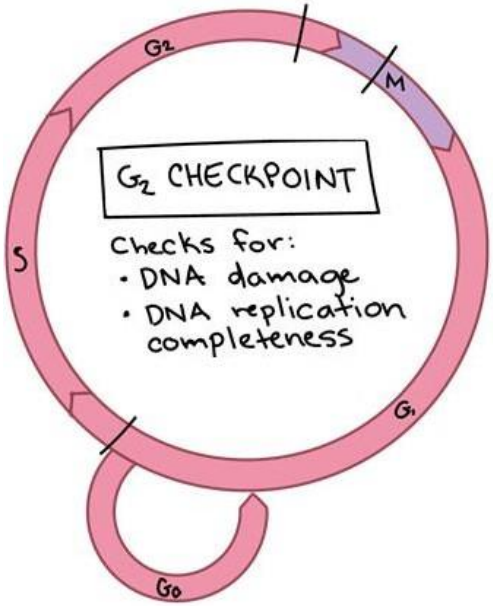


How do the checkpoints actually work?

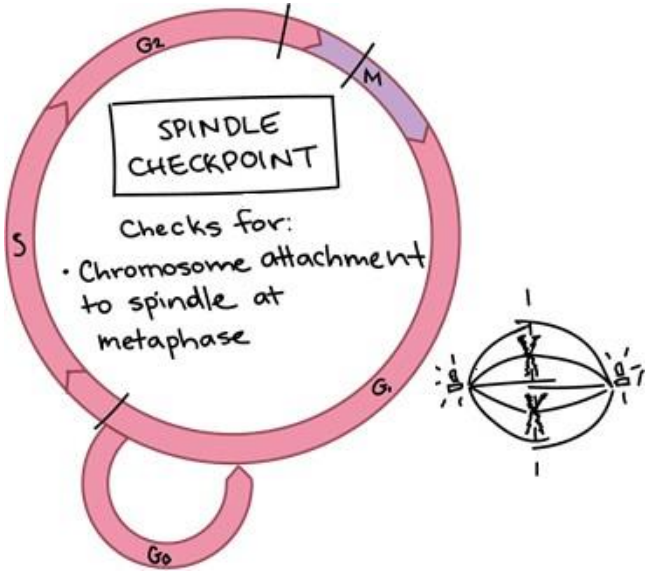
The checkpoints



The **G-1 checkpoint** is the main decision point for a cell – that is, the primary point at which it must choose whether or not to divide.

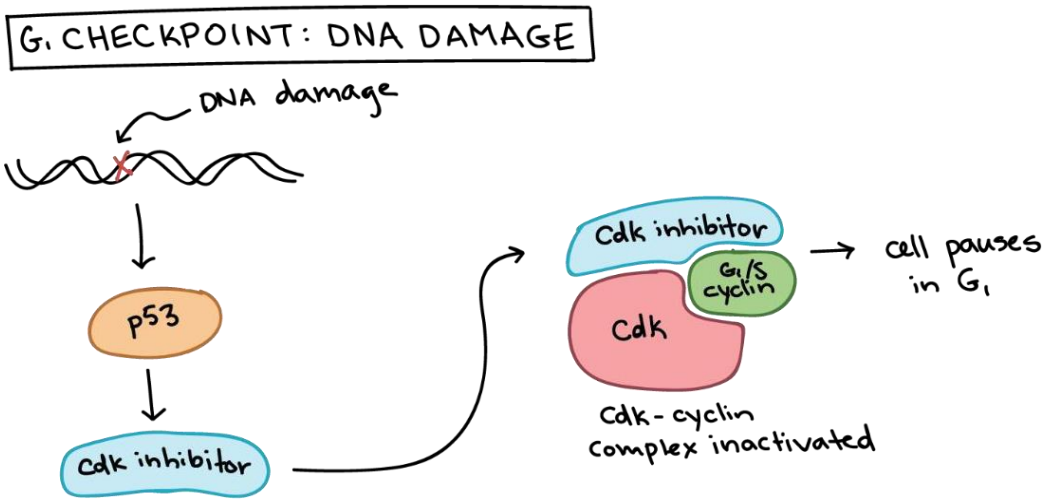


Once the cell passes the **G-1 checkpoint** and enters S phase, it becomes irreversibly committed to division.

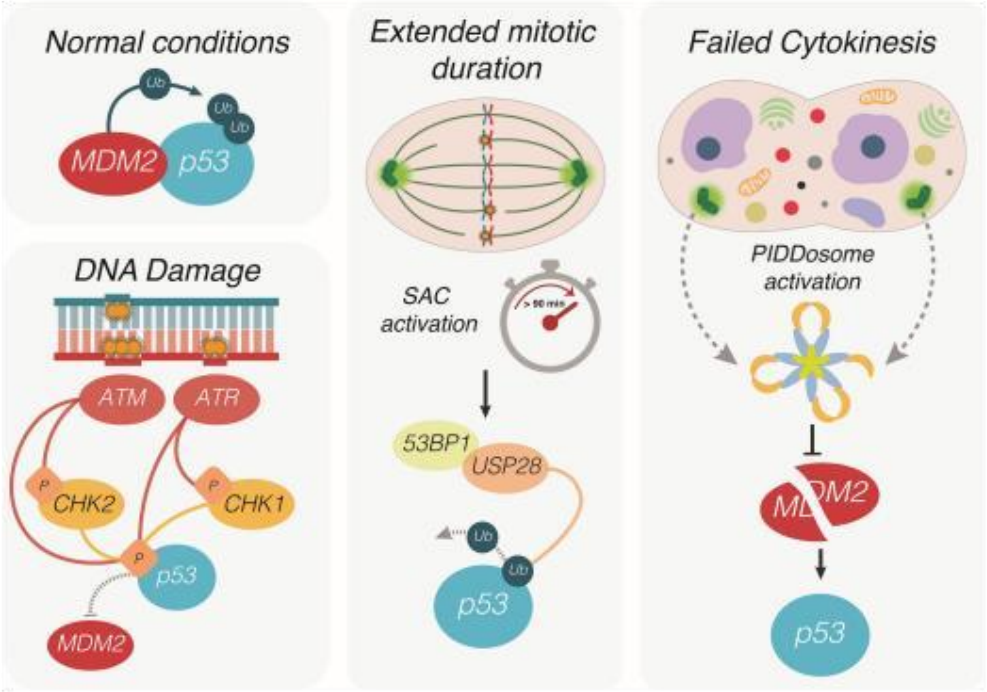


Checkpoints and regulators

- p53 works on multiple levels to ensure that cells do not pass on their damaged DNA through cell division.
- **First:** it stops the cell cycle at the G- 1 checkpoint by triggering production of **Cdk inhibitor** (**CKI**) proteins. The CKI proteins bind to Cdk-cyclin complexes and block their activity, buying time for DNA repair.
- **Second:** to activate DNA repair enzymes.
- **Third:** If DNA damage is not fixable, p53 will play its **final role by** triggering programmed cell death so damaged DNA is not passed on.



Different routes leading to p53 activation.



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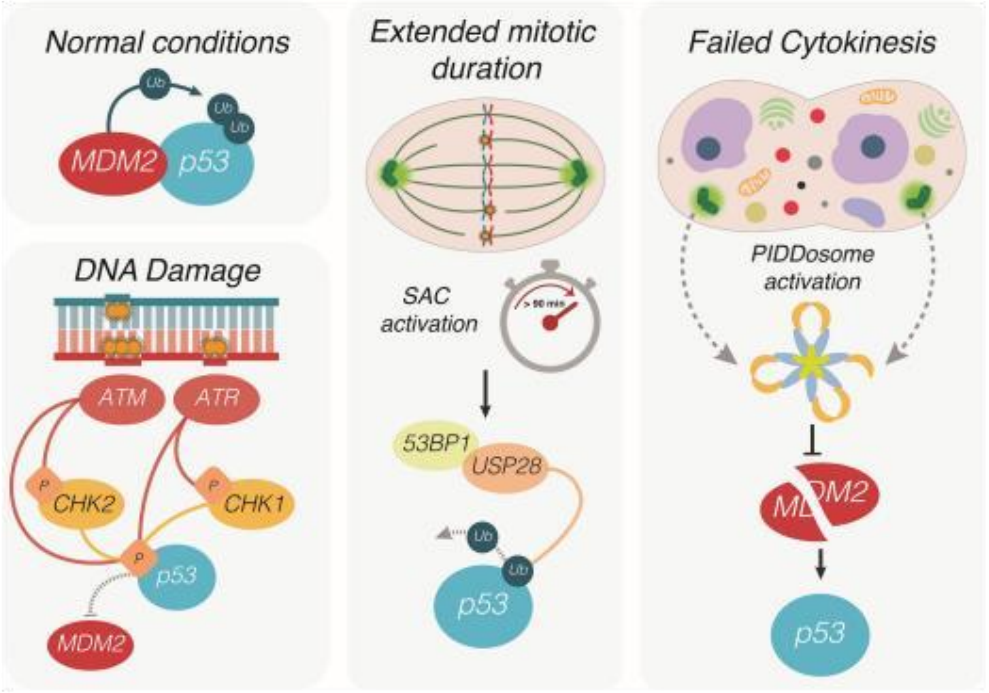
- **1- In physiological conditions,** p53 is bound and ubiquitinated by its negative regulator MDM2, which prevents nuclear translocation and promotes its proteasomal degradation.
- Upon single strand or double strand DNA damage, the kinases ATR and ATM activate the checkpoint kinases Chk1 and Chk2, which contribute to p53 phosphorylation on specific amino acidic residues. Phosphorylated p53 can no longer be bound and degraded by MDM2, resulting in protein stabilization, nuclear translocation and the activation of its transcriptional program

Different routes leading to p53 activation.



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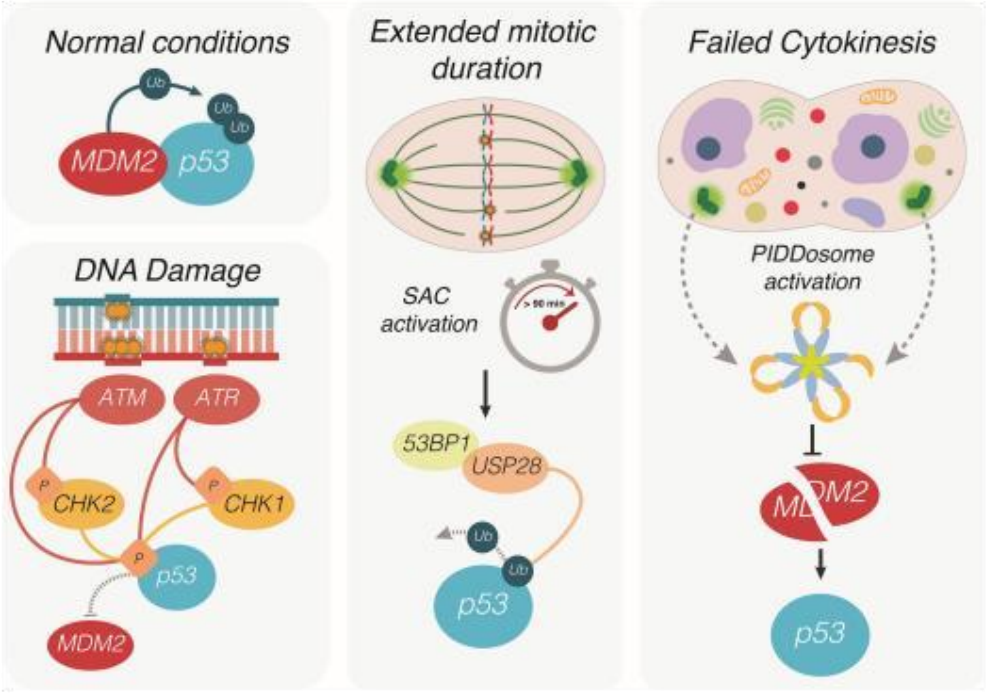
- **<u>2- During mitosis,</u>** prolonged prometaphase due to the activation of the spindle assembly checkpoint (SAC) is sensed by 53BP1 and USP28. The latter protein promotes de-ubiquitination of p53, arresting the cell cycle in the next interphase.

Different routes leading to p53 activation.



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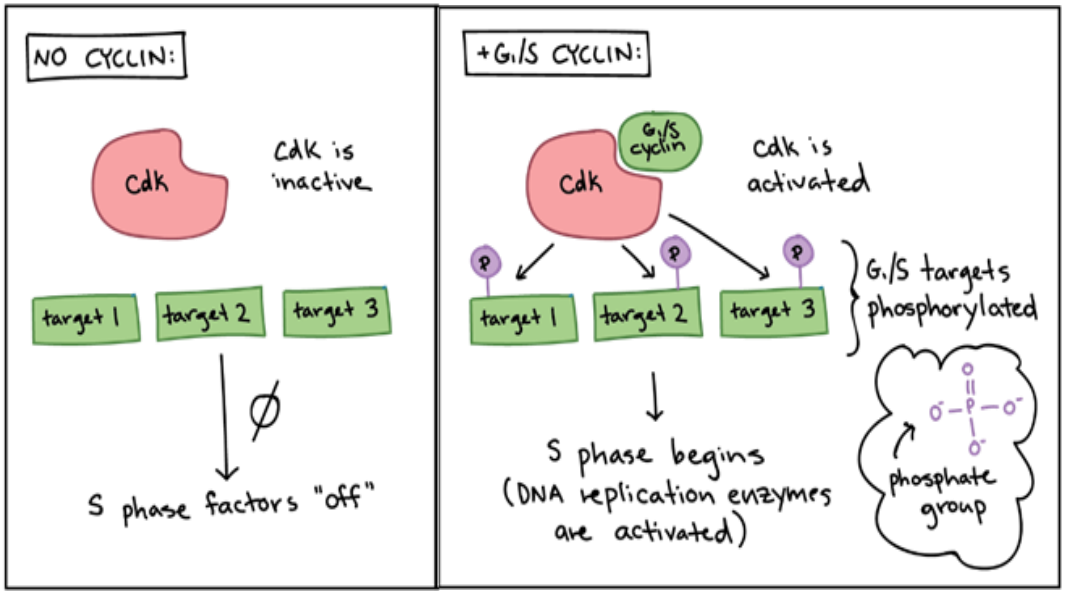
- **<u>3- Defective cytokinesis</u>** prevents daughter cells to separate completely at the end of mitosis, resulting in a single polyploid cell containing extra centrosomes. The multiprotein complex PIDDosome senses the presence of extra centrosomes and functions as an activating platform for caspase-2. Being a target of this protease, MDM2 is cleaved and thereby inactivated, resulting in the accumulation of p53 and cell cycle arrest of the polyploid cell.

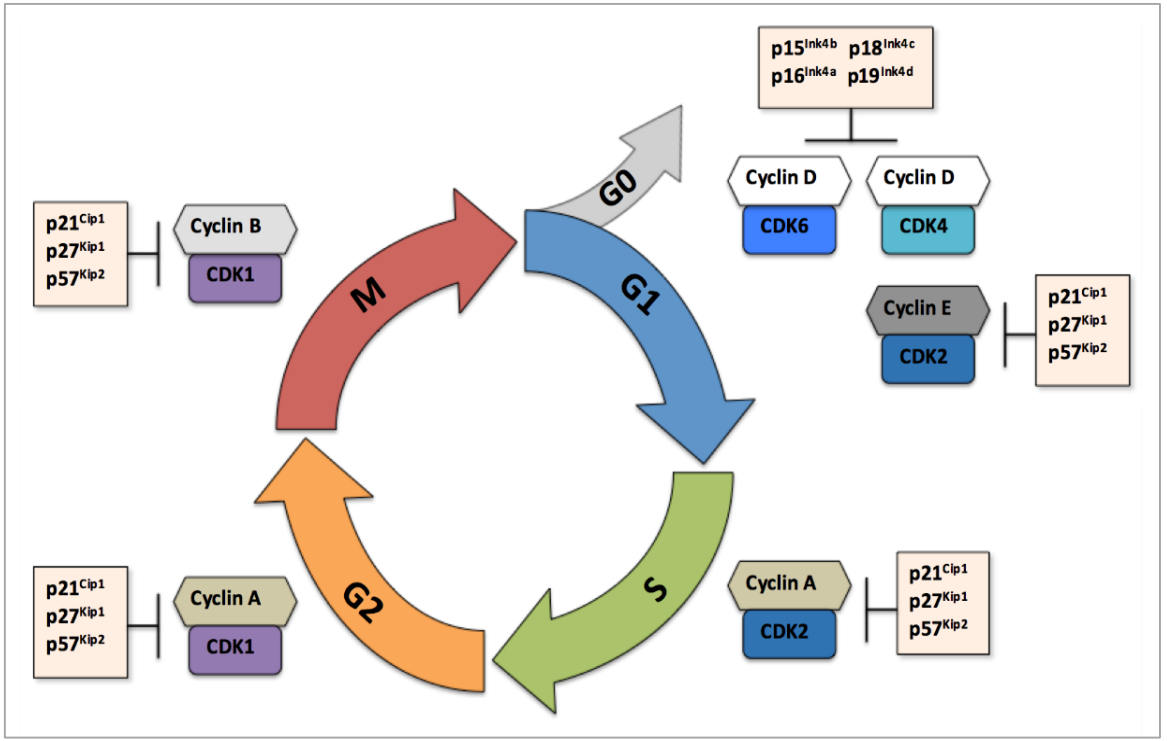
Cyclins and cyclin-dependent kinases (cdks)

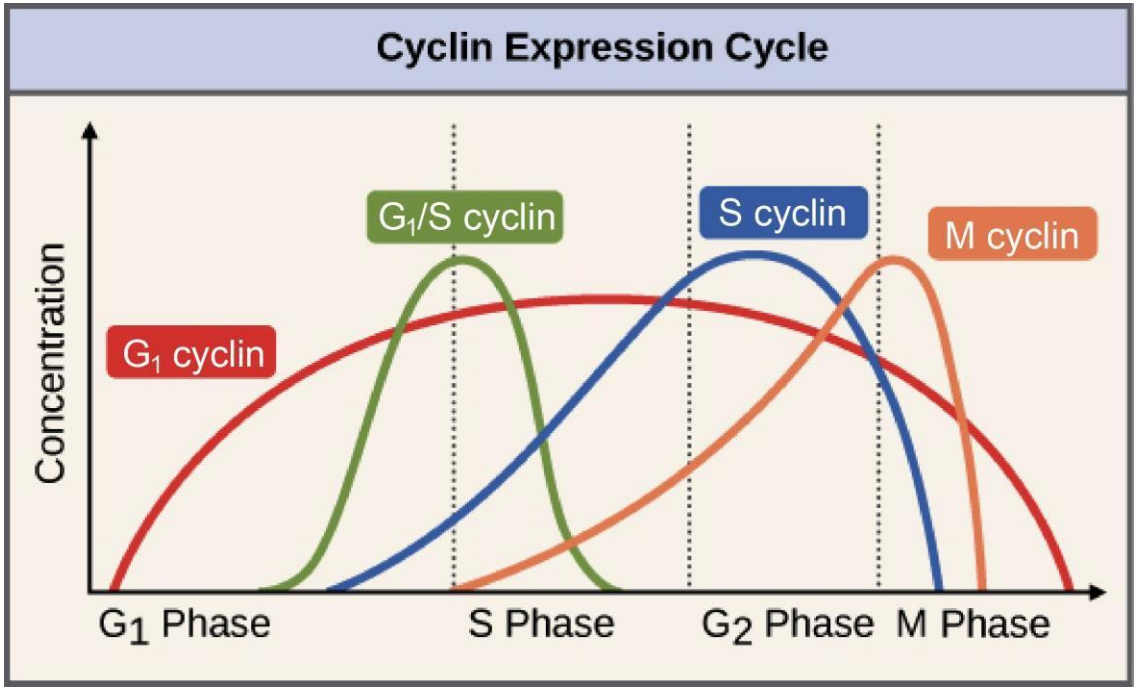
- Most cells in an adult are not in the process of cell division. They are quiescent and

  enter an inactive period G0, a phase outside of the cell cycle.
- Mitogens or growth factors can, however, induce cells in G0 to re-enter the cell cycle and pass the G1 restriction point.
- The passage of the cell through the different phases of the cell cycle is coordinated and regulated by a set of proteins called cyclins and their associated cyclin-dependent kinases (cdks).



- The pairing of cyclins to the cdks is highly specific. Upon binding of a cyclin to its cdk partner, cyclin induces a conformational change in the catalytic subunit of the cdk revealing its active site.
- Different cyclin–cdk complexes are present at specific points in the cell cycle and are important regulators of irreversible phase transitions.
- The cyclin–cdk complexes exert their effect by phosphorylating target proteins.





- **Cyclins were originally named because their concentration varies depending on the transcription of their genes and by subsequent regulated protein degradation. The concentration of cdks does not fluctuate during the cell cycle.**
- **The concentrations of cyclin proteins change throughout the cell cycle.** There is a direct correlation between cyclin accumulation and the three major cell cycle checkpoints. M cyclin, for example, peaks dramatically at the transition from G2 to M phase. G_1 cyclins are unusual in that they are needed for much of the cell cycle.

Mechanisms of cdk regulation

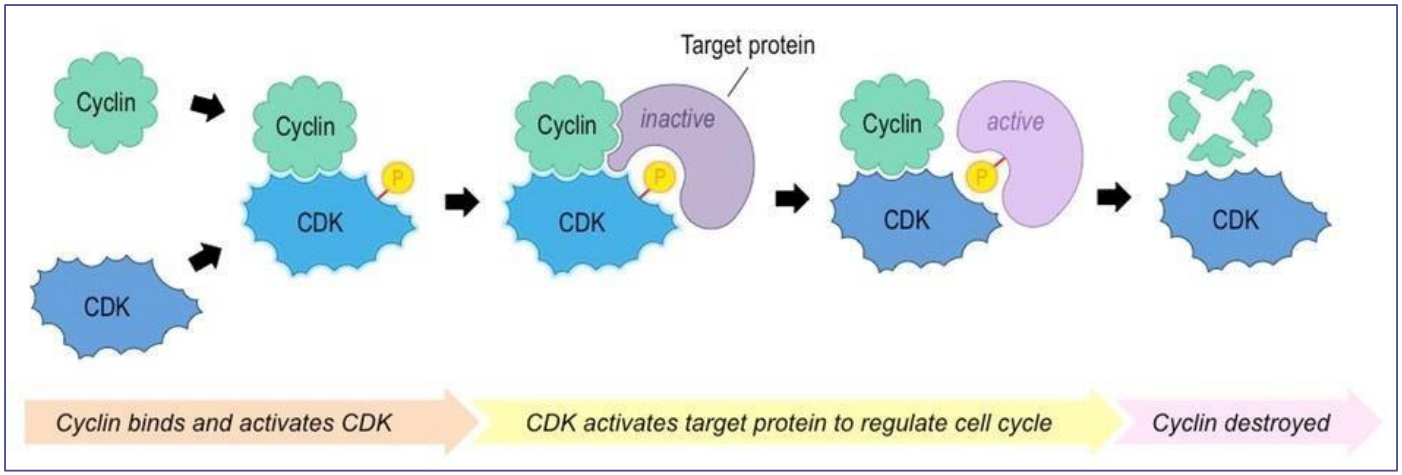
- Cdks are serine/threonine kinases that, sequentially, regulate progression through the phases of the cell cycle via phosphorylation. Therefore, the regulation of cdk activity is crucial for precise cell reproduction.
- There are four mechanisms of cdk regulation:
  1. association with cyclins,
  2. association with cdk inhibitors,
  3. addition of phosphate groups that activate cdk activity, and
  4. addition of phosphate groups that inhibit cdk activity

What are the four mechanisms of cdk regulation?

Because of the precise window of time for which regulators of the cell cycle are required, the ‘disappearance’ of a factor is as important as its appearance. That is, precise protein degradation also plays an important role in the control of the cell cycle

1. Association with cyclins

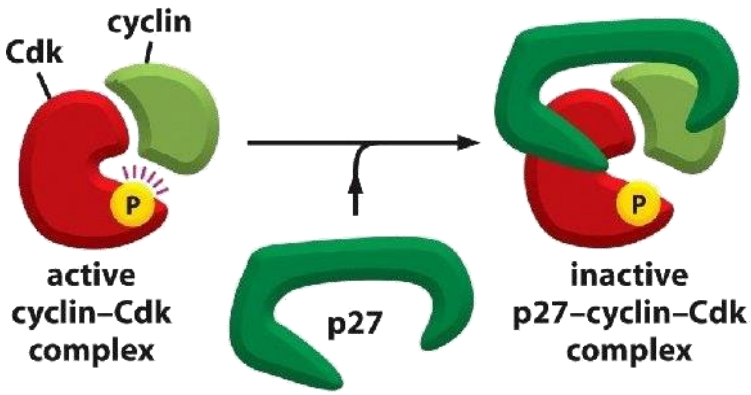
- The binding of cyclins to their partner cdk causes a crucial conformational change in the cdk that allows binding of a protein substrate and correct positioning of ATP. The inactive cdk molecule has a conformation that blocks the binding of the protein substrate and correct alignment of ATP.
- Ubiquitin-mediated proteolysis of cyclins prevents the constitutive activity of cdks.



2. Association with inhibitors

- The p21 inhibitors interact **with both** cyclins and their associated cdks (mainly with cdk2 and cyclin E) and block the ATP-binding site, thus disabling kinase activity.

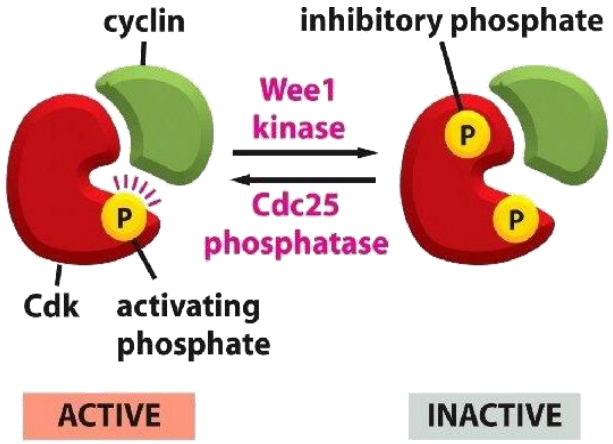
- Again, ubiquitin-mediated degradation of inhibitors ensures that the inhibitors are present during a defined window of time during the cell cycle.



The inhibition of a cyclin-Cdk complex by a CKI

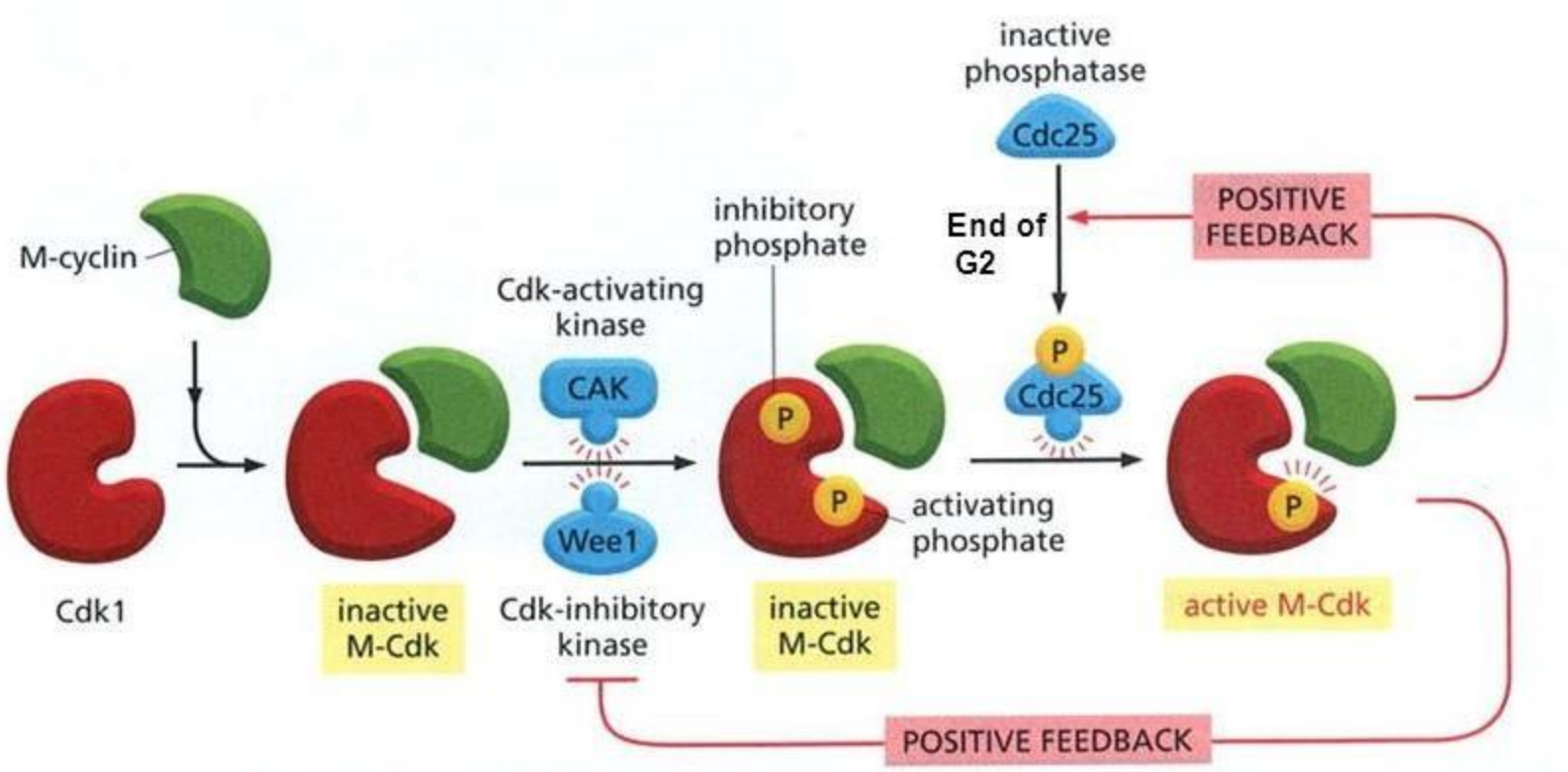
3. Regulation by phosphorylation

- Regulation of cdk activity by phosphorylation involves both activation and inhibition. Two phosphorylation sites on the amino-terminal end are inhibitory when phosphorylated. The tyrosine kinase, wee1, phosphorylates Thr14 and Tyr15.
- Two steps are required for cdks to become active: dephosphorylation of the inhibitory phosphate groups by cdc25 phosphatases and phosphorylation of a central threonine residue, Thr161, by cdk-activating kinase (CAK).



The regulation of Cdk activity by inhibitory phosphorylation

Activation of M-Cdk

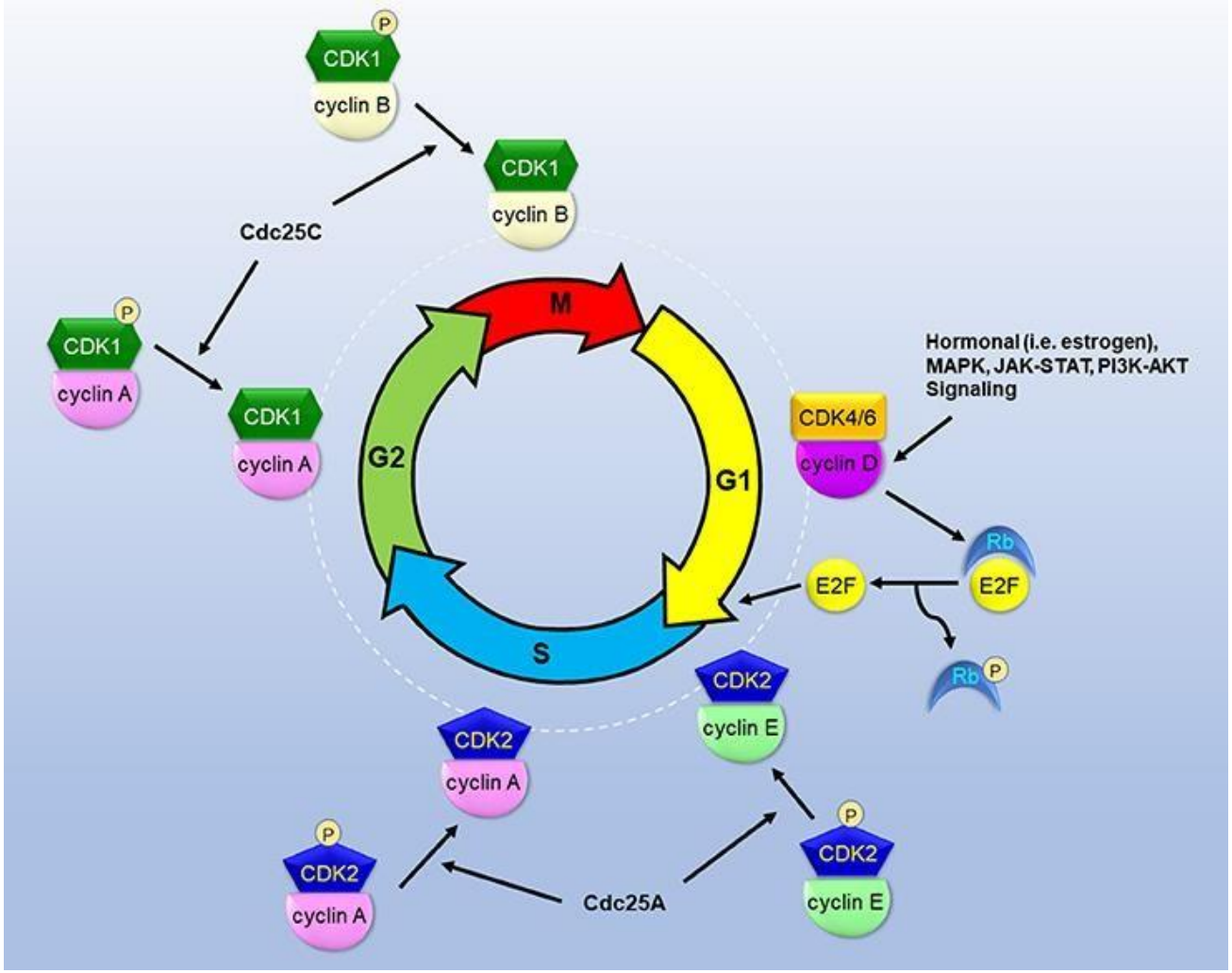


Why understanding cell cycle regulation is so important?

Cell cycle control and cancer	
Cyclin D1	Cyclin D1 Amplification in 20% Mammary cancer Translocation / amplification in 50% of lymphoma Cyclin D mRNA and protein levels are over-expressed in 50% of breast cancers.
Cdk4	Overexpression in mammary cancer and glioblastoma
Cyclin E	Overexpression in 10% mammary cancer
Cdc25	Overexpression in 20+ cancers
p16	Loss (non-mutational) in many cancers Mutation in inherited melanoma
p27	Loss in many cancers Mutation in inherited neuroendocrine cancers
Rb1	Loss / mutation in cancers (retinoblastoma, osteosarcoma)
Chk2	Mutation in Osteosarcoma

Genes encoding cell cycle regulators are frequently mutated in human tumors leading to aberrant regulation of the cell cycle, unscheduled proliferation, and carcinogenesis

Example…. RB/E2F



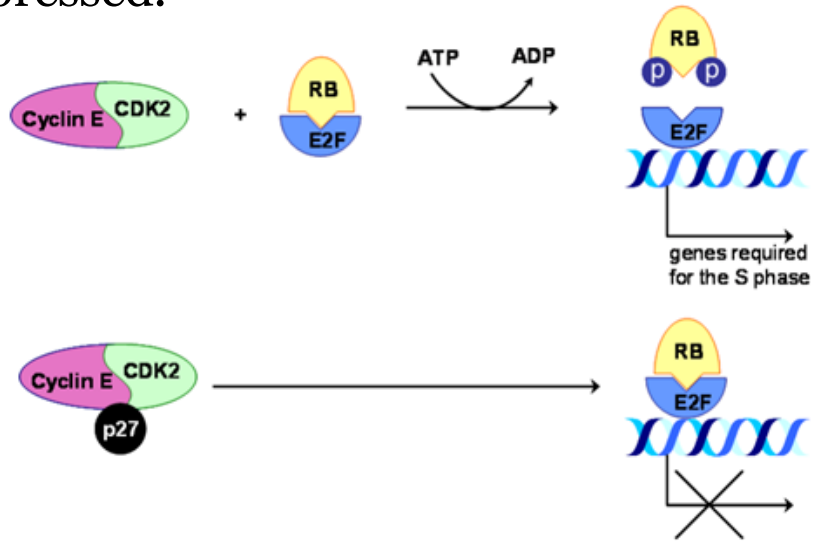
Molecular mechanisms of the effects of RB

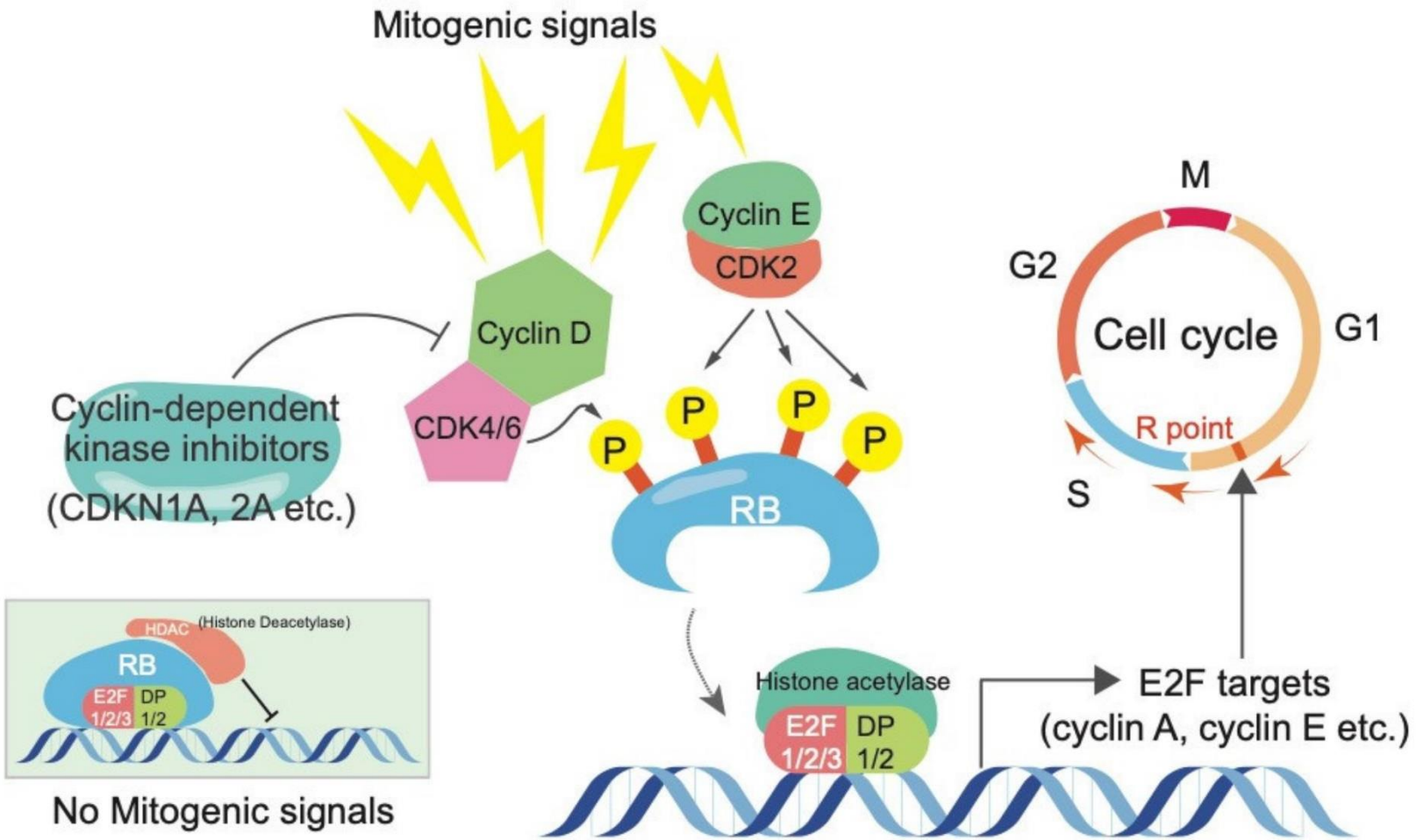
- The primary substrate for D-type cyclin kinases is the **retinoblastoma tumor suppressor protein (Rb).**

- The major point of control for RB protein is the transition from the **G1 phase of the cell cycle to S phase.**

- RB does not bind to specific DNA sequences but instead regulates the activity of the E2F transcription factor family, which is crucial for the expression of genes needed for the S phase.
- In cells lacking Rb, D-type cyclin kinase activity is not required for cell cycle progression.

- The interactions between RB and E2F and HDACs are **regulated by serine/threonine phosphorylation**. In the absence of a growth signal, RB is in a hypophosphorylated state and binds to both E2F and HDAC.
- By binding to E2F, RB sequesters it and blocks its transactivation domain, preventing E2F from interacting with the general transcription factors.
- Thus, the trimeric complex of RB with HDAC and E2F regulate transcription and, consequently, cell cycle progression; genes such as cyclin E, cyclin A, and cdk 2, whose products are required for progression through the cell cycle, are not expressed.




Mitogenic signals
Cyclin E
CDK2
Cyclin D
CDK4/6
Cyclin-dependent
kinase inhibitors
(CDKN1A, 2A etc.)
P
P
P
P
RB
M
G2
Cell cycle
G1
R point
S
HDAC
(Histone Deacetylase)
RB
E2F
1/2/3
DP
1/2
No Mitogenic signals
Histone acetylase
E2F
1/2/3
DP
1/2
E2F targets
(cyclin A, cyclin E etc.)

Therapeutic strategies

- Cyclin-dependent kinase inhibitors

- The targeting of the cell cycle presents unique opportunities for drug development in cancer therapy.
- Phosphorylation by cdks is a key step in the regulation of the cell cycle. These serine/threonine kinases are over-expressed and/or amplified in some cancers, making them possible molecular targets for cancer therapies. A semi-synthetic flavonoid called flavopiridol acts as a competitive inhibitor of all cdks tested, by targeting their ATP-binding site.
- Flavopiridol induces cell cycle arrest at G1/S and G2/M phases.

Flavopiridol

- Exposure of MDA-MB-468 human breast cancer cells to flavopiridol for 72 hours induces cell cycle arrest. In other cell lines (e.g. U937 cells) it has been shown to induce apoptosis.
- As a single agent flavopiridol has been shown to be completely ineffective in the treatment of the HCT-116 colon cancer cells. In vitro flavopiridol induced growth arrest but no cell death in the HCT-116 cells.
- When combined with irinotecan (CPT-11), flavopiridol significantly enhanced CPT-11 induced apoptosis in vitro and potently augmented the response to CPT-11 in vivo.
- In fact, this resulted in pathologic cures that were not achievable with CPT-11 alone. This was highly sequence specific such that CPT-11 needed to come before flavopiridol in order to induce this effect .

- An understanding of the cell cycle will ultimately determine the success of this class of drugs, especially when combined with chemotherapy. Too early treatment with a CDK inhibitor may block a tumor cell at a phase of the cell cycle that is not favorable to the induction of apoptosis.
- E.g. treatment of MDA-468 breast cancer cells with flavopiridol

- Other cell cycle kinase targets

- Cell cycle checkpoint kinase inhibitors (e.g. against Chk1 and Chk2) are also being identified and used as an anticancer strategy. These agents prevent cell cycle arrests and may potentiate the effects of classical chemotherapeutics that cause DNA damage and subsequent apoptosis.