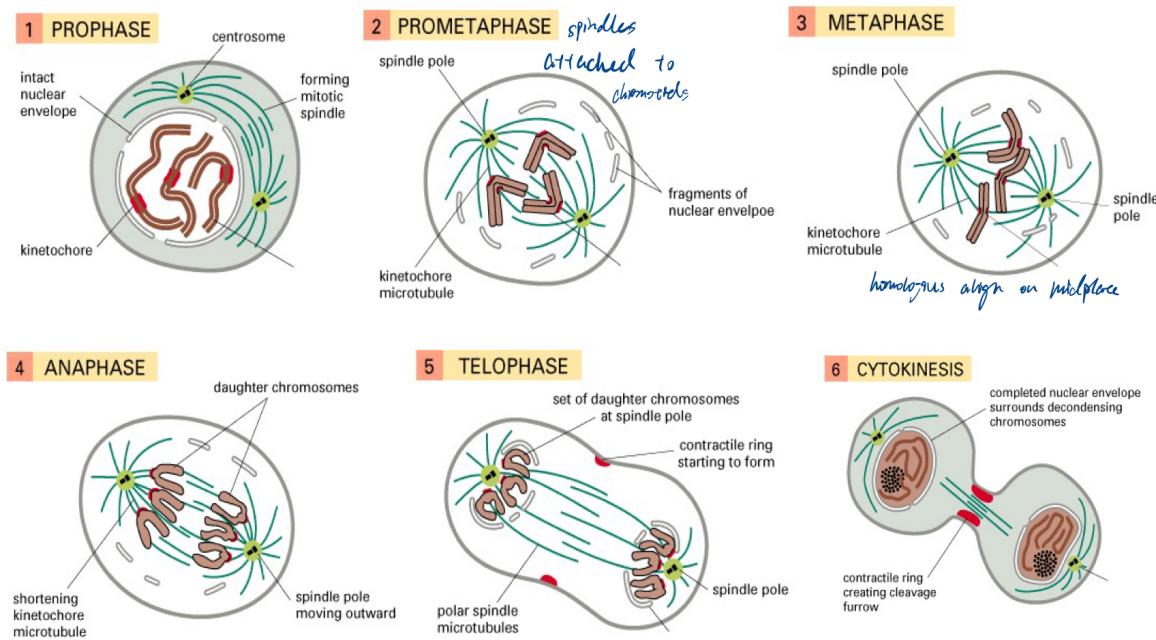


Cell cycle

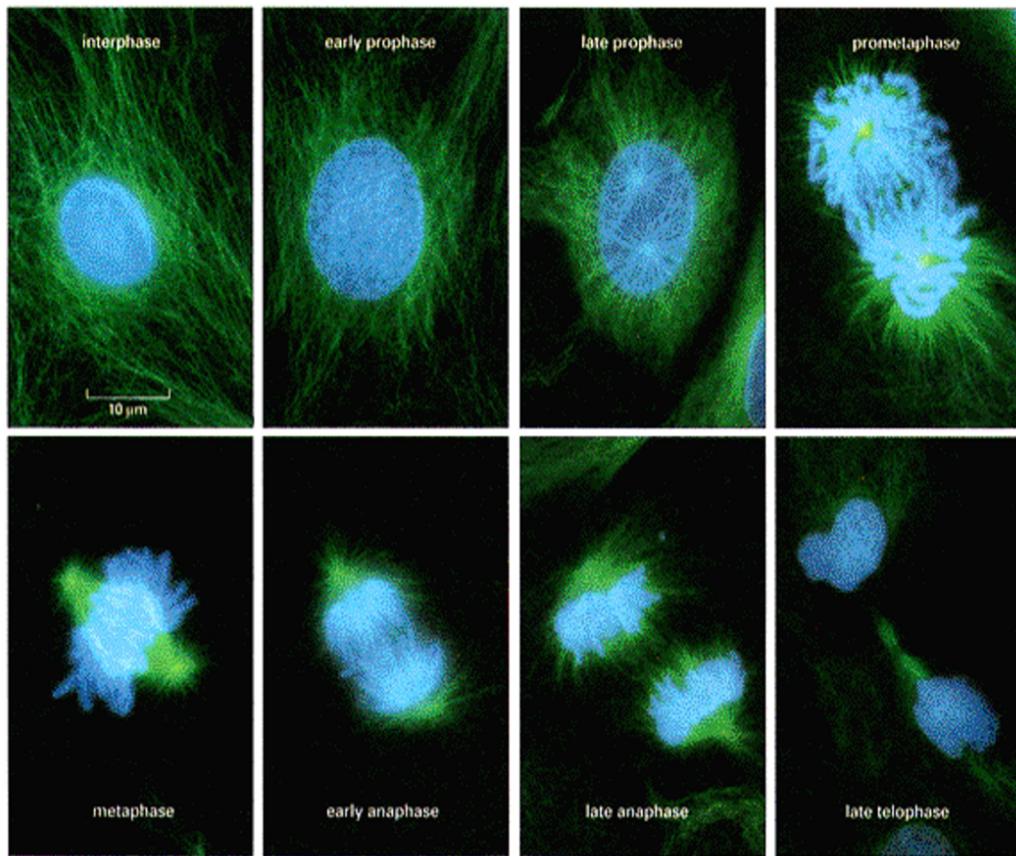
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▼ Mitosis

- replicate only once
- timely-manner
- accurate

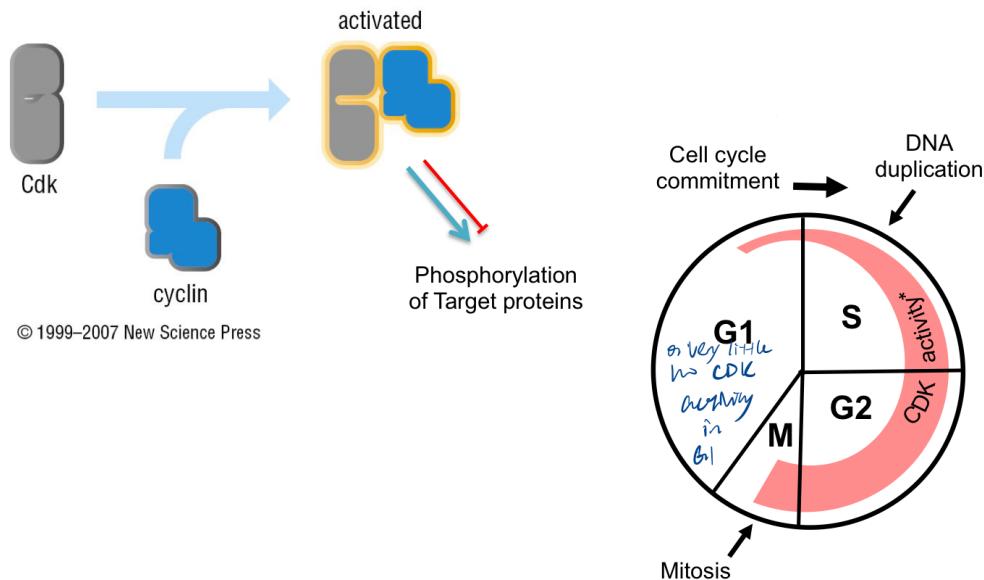


Chromatin:blue, Microtubules:green



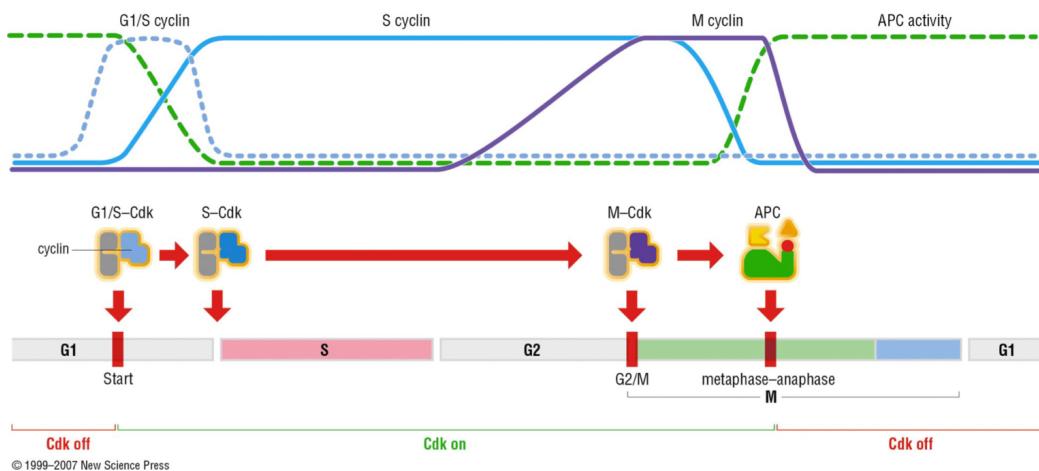
The Cell Cycle; Cyclin-Dependent-Kinase* (CDK)

*Kinases can add a phosphate group to another molecule



The Cell Cycle; CDK activity drives the cell cycle

This cyclin whose regulation, or there



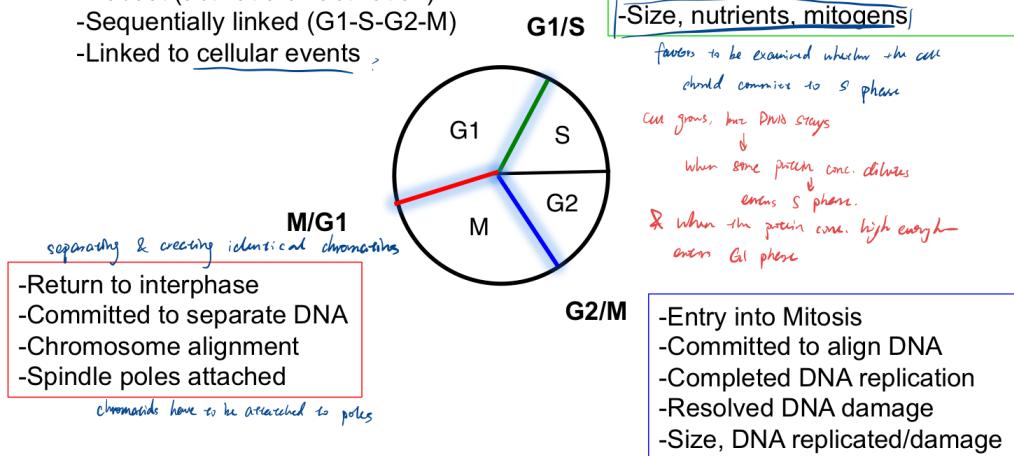
- overall, different cyclin activities are increased at different stages; there is an increased overall cyclin kinase activity starting from G1 phase

▼ Three decision window

Cell Cycle Control Dynamics; Cell Cycle Transitions

Cell Cycle Transition Pathways

- Unidirectional (^{otherwise ie. entry back & re-enter more} non-reversible)
- Robust (activation/inactivation)
- Sequentially linked (G1-S-G2-M)
- Linked to cellular events



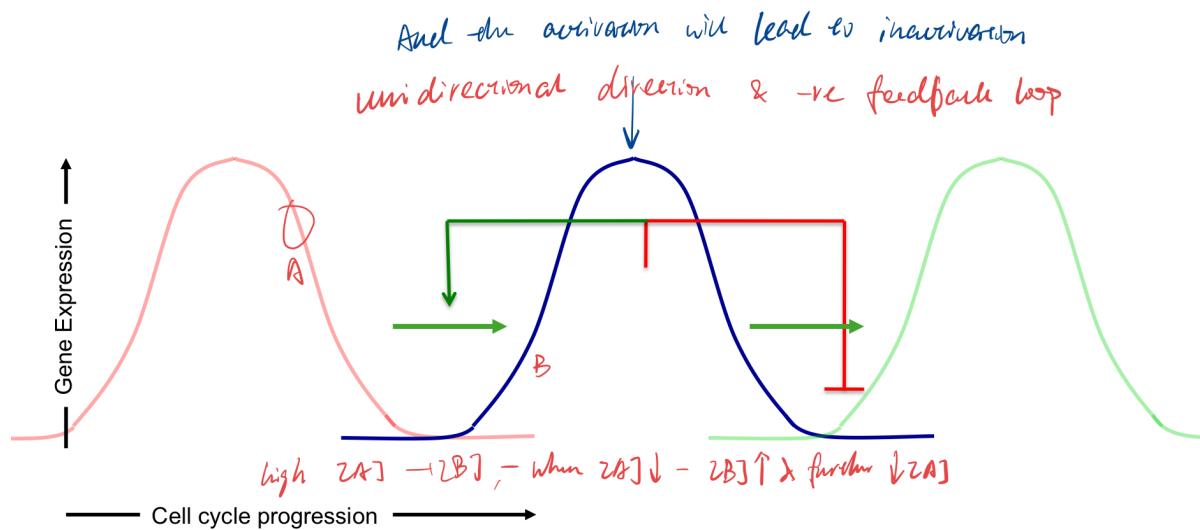
1. Transitioning from G1 to S phase (in G1 phase) G1/S
 - a. commit to initiate DNA replication → cell division
 - b. otherwise would exit the cell cycle to a non-proliferative state - quiescence G0
 - c. depending on cell size; nutrients; mitogens etc
2. Entry of M phase (in G2 phase) G2/M
 - a. whether the DNA replication is completed - to properly align and separate chromatids
 - b. whether there are DNA damage
 - c. Resolve the DNA damage
 - d. cell size
3. Exit M phase and reenter interphase M/G1
 - a. correct alignment of sister chromatids - to separate DNA to form two genetically identical cells

- b. Are two spindle poles attached & are chromatids attached to the spindles of correct directions

Transcriptional control of the cell cycle

▼ CDK activity and APC/C activity are central to the control of cell cycle progression

- Driven by the accumulation of **cyclin-dependent kinase activity** from interphase to early M phase
- **APC/C activity** mediates its loss of activity during late M phase (with CDC20) and entering G1 phase (with CDH1) → **marking the return back to interphase**
- These can be perceived as consequential waves:

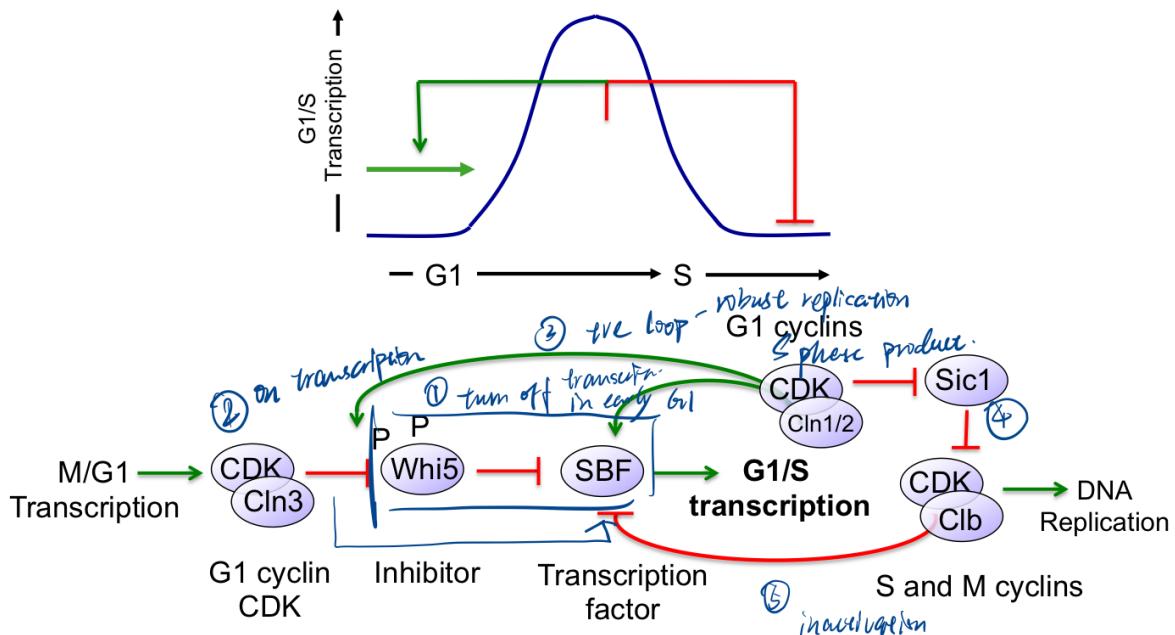


for a new wave to initiate - the previous wave must be completed first

Example of Yeast cycle

G1/S transcription initiates the G1 to S transition

Robust and committed activation linked to inactivation: Feedback!

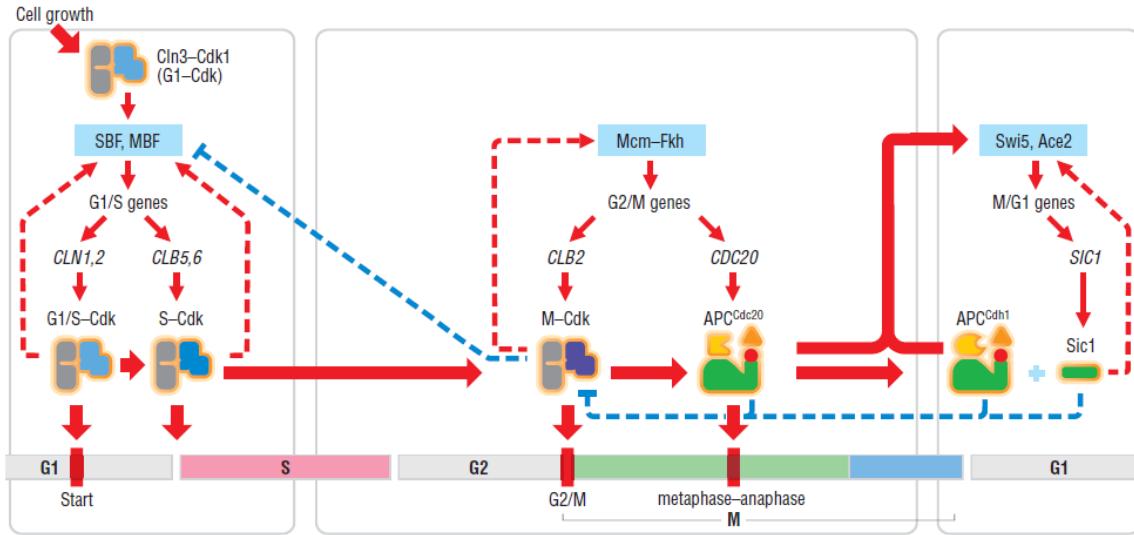


G1 transcription inhibited in G1 (Whi5 → SBF)

When can you complete M & enter G1: $\frac{CDK}{Cln3} \xrightarrow{P} Whi5 \rightarrow SBF$

G1/S transcription producer: $\frac{CDK}{Cln2} \rightarrow Whi5$ ensure it's robust
 $\frac{CDK}{Cln2} \rightarrow SBF$ & unidirectional
& irreversible

When enter S phase: $\rightarrow Sic1 \rightarrow \frac{CDK}{Clb}$
 $\rightarrow \frac{CDK}{Clb} \rightarrow DNA \text{ replication}$



- During early G1: **Whi5 suppresses SBF** (SCB-binding factor) and MBF (MCB-binding factor), it prevents those TF to bind on DNA promoter site to initiate transcription
- Late G1: accumulation of **Cln3-Cdk1** → **inhibitory phosphorylation of Whi5** → unleash the active SBF and MBF → express genes encoding **G1/S cyclins: Cln1 and Cln2 & S cyclins :Clb5 & Clb6 & enzymes required for replication and other S events** → ready to begin S phase
- accumulation of Cln1/2-Cdk acts as a positive loop to further inhibit Whi5 activity and further upregulate expression of G1/S & S cyclins → **makes S phase more robust and unidirectional**
- Progression of **S phase** promotes the formation of Ndd1 complex (**Mcm-Fkh-Ndd1**) → stimulate expression of **G2/M mitotic regulatory proteins**: mitotic entry → **M cyclin Clb2**; mitotic exit **APC activator Cdc20**
- As the cell progressing the M phase: active **M-Cdk (activated by Clb2)** **then further enhances the activity of APC/Cdc20** → to a certain level **it then inhibits the M-Cdk** activity → exit M phase
- **APC/Cdc20** activates **Swi5 & Ace2** → prepare for M/G1 genes for further M exit → **accumulation of Sic1** → **inhibit M-Cdk (ClnB-Cdk)** → stop M phase
 - ClnB-Cdk in M phase inhibits SBF & MBF for S phase

→ Upregulation of **Sic1** also help regeneration of SBF and MBF for a new cycle of replication

- Note the positive feedback loop:
 - G1/S-Cdk (Cln1/2) & S-Cdk (Cln 5/6)
 - M-Cdk (ClnB)
 - Sic1

▼ Replication: **timely manner ; robust ; only once — done by licensing**

- Only once - licensing
- licensing
 - At early G1: **in the absence of Cdk activity / high APC/C activation** state → **Mcm** helicases can bind upstream to the replication origins (and to load origin recognition complex (ORC) to replication origins)
 - This process is called licensing, happens only in the absence of Cdk activity
- Replication requires the activation of Mcm activity
 - Mcm is activated only in the presence of **S-Cdk activity**
- Timely manner & robust
 - Bidirectional
 - Many replication origins

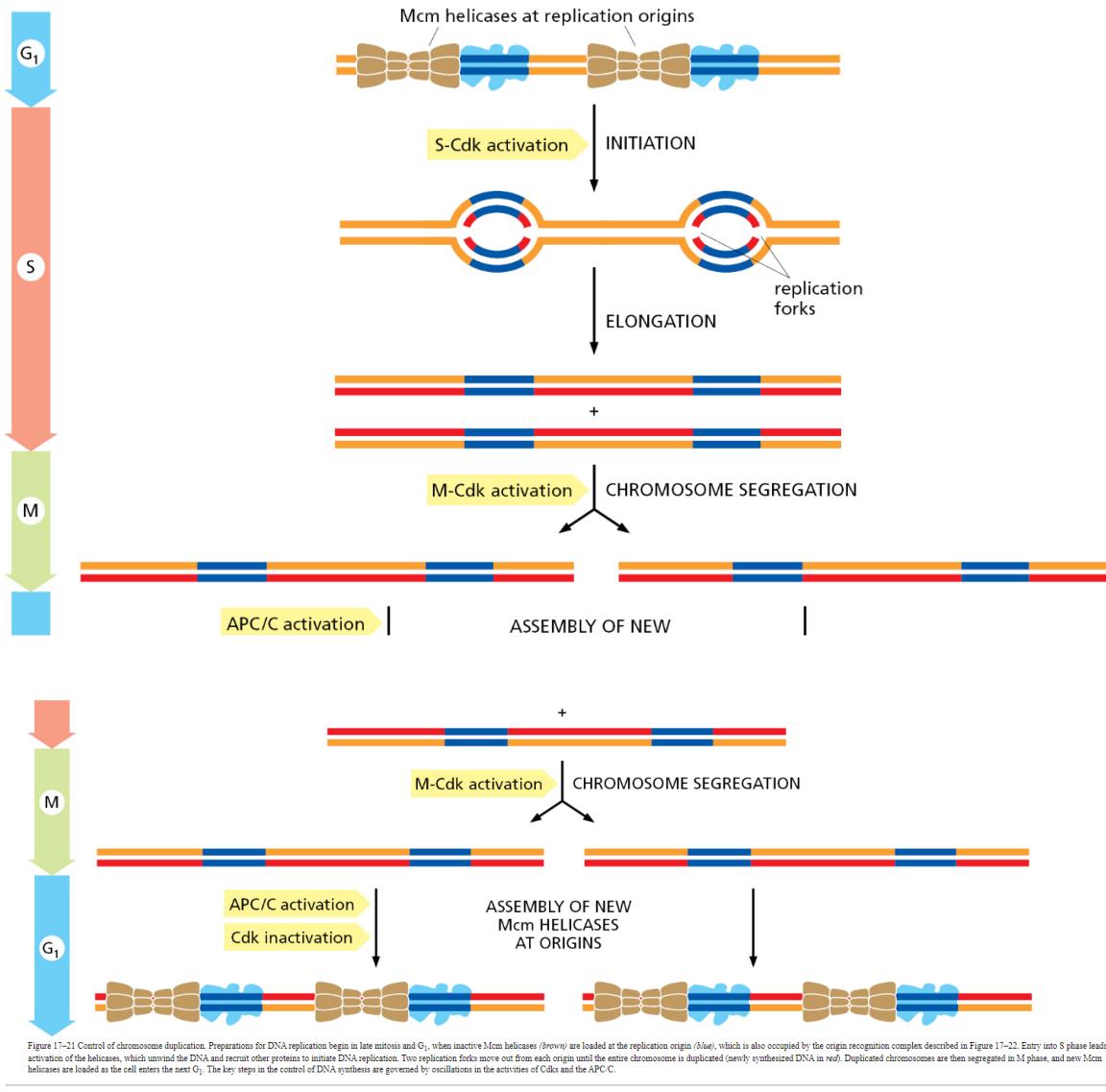
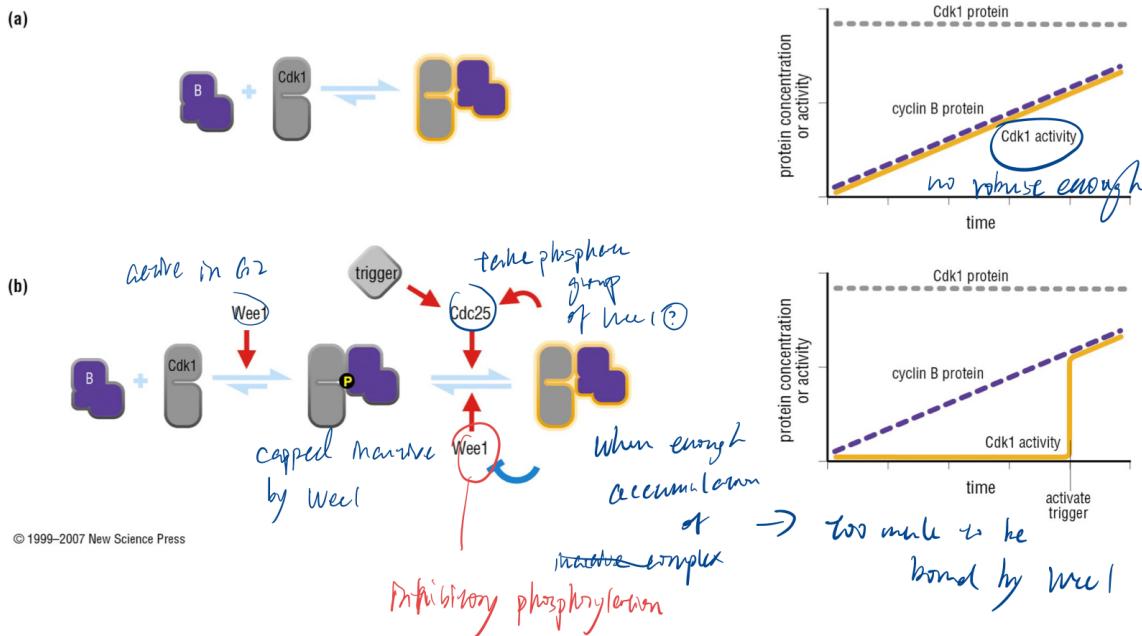


Figure 17–21 Control of chromosome duplication. Preparations for DNA replication begin in late mitosis and G₁, when inactive Mcm helicases (brown) are loaded at the replication origin (blue), which is also occupied by the origin recognition complex described in Figure 17–22. Entry into S phase leads to activation of the helicases, which unwind the DNA and recruit other proteins to initiate DNA replication. Two replication forks move out from each origin until the entire chromosome is duplicated (newly synthesized DNA in red). Duplicated chromosomes are then segregated in M phase, and new Mcm helicases are loaded as the cell enters the next G₁. The key steps in the control of DNA synthesis are governed by oscillations in the activities of Cdks and the APC.

▼ Mechanism to achieve the positive feedback — different from self-activation & cyclin B - Cdk1



- Wee1 is a kinase - can phosphorylate enzymes
- Cdc25 - phosphatase - remove phosphate group
- When [cyclin B] is low: Wee1 conc. is high vs Cdc25 conc. is kept low
- When [cyclin B] ↑ — cyclin B has high affinity to Cdk1 — **Wee1 phosphorylates** the cyclinB-Cdk1 complex → inhibitory phosphorylation
- Potential activating mechanism (cyclin A-Cdk2 in G2) → partly activate phosphatase **Cdc25**
- remove the phosphate group → Cdk1 activity increases
- **Cdk1 itself can activate more Cdc25 & inhibit Wee1: main reason for the robust rise in activity**
- Once activated, Cdk1 remains active even without trigger stimuli
- Cdk activation is generally governed by multiple overlapping mechanisms: the rise in cyclinB-Cdk1 activity promotes expression of cyclin B

▼ Regulation of APC activity

- APC is essential for transition from **metaphase to anaphase**
- APC — anaphase-promoting complex (APC): **ubiquitin-protein ligase**

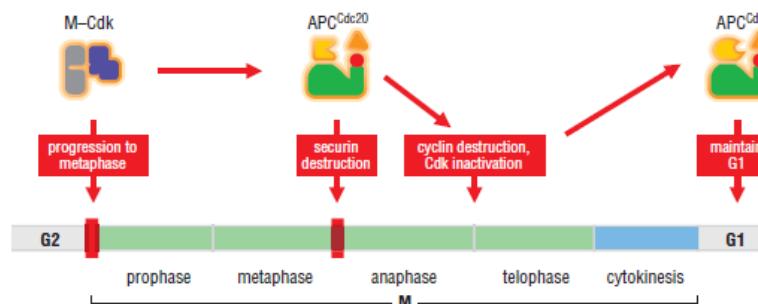


Figure 3-28 Control of late mitotic events by the APC M-Cdk activity promotes the events of early mitosis, resulting in the metaphase alignment of sister chromatids on the spindle. M-Cdk activity also promotes the activation of APC^{Cdc20}, which triggers anaphase and mitotic exit by stimulating the destruction of regulatory proteins, such as securin and cyclins, that govern these events. By promoting cyclin destruction and thus Cdk inactivation, APC^{Cdc20} also triggers activation of APC^{Cdh1}, thereby ensuring continued APC activity in G1.

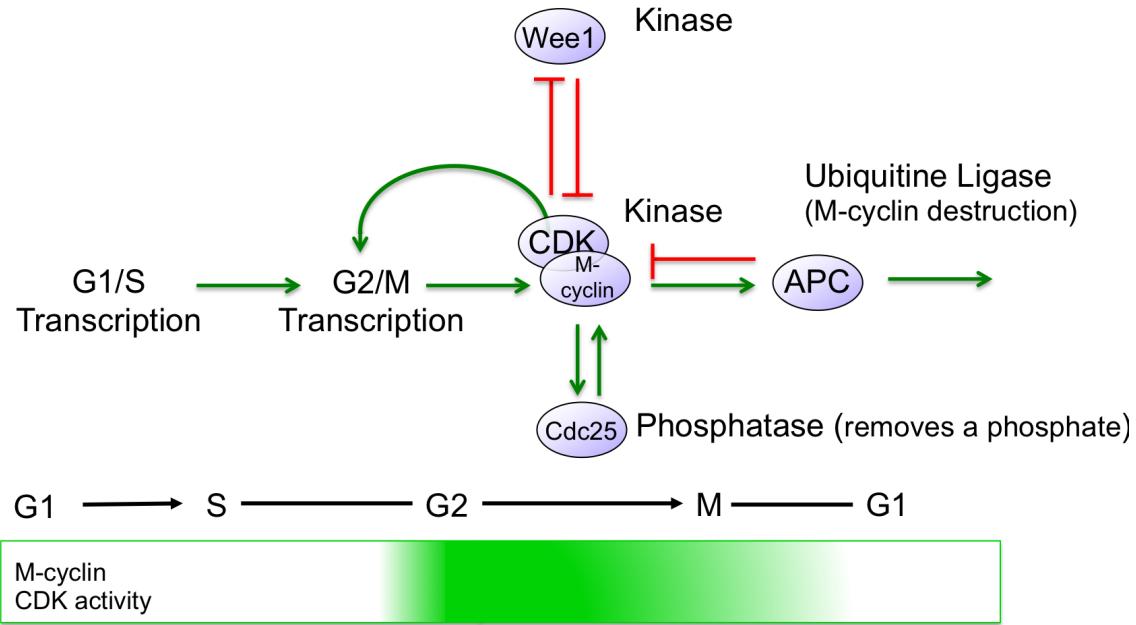
▼ APC is activated by two activator subunits

▼ Cdc20

- activates APC during metaphase-anaphase transition

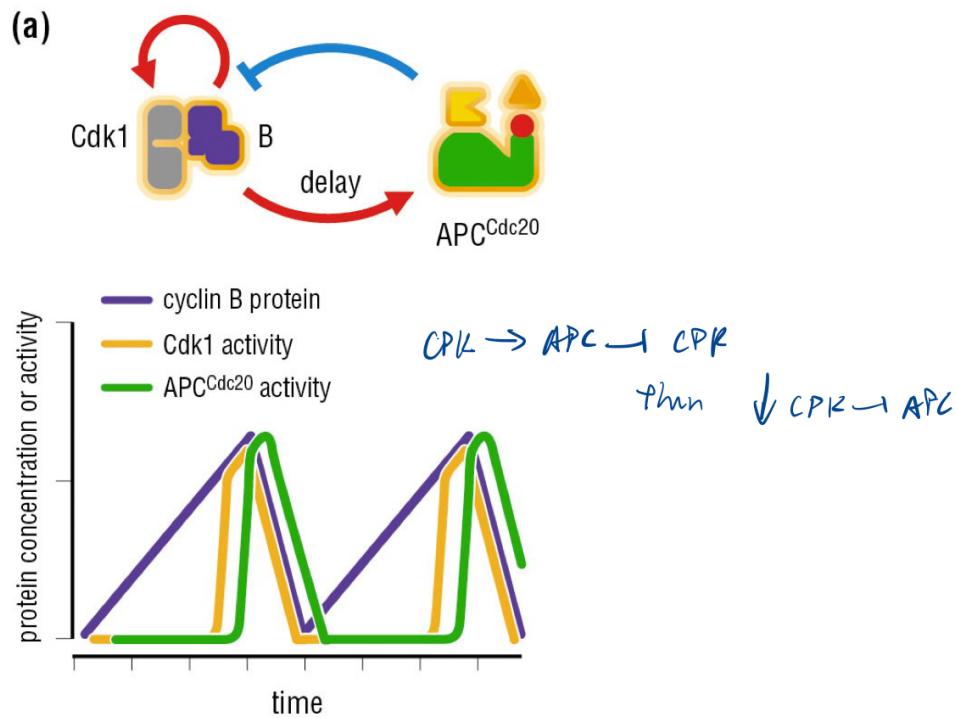
▼ Cdh1

- activates APC at late M and early G1 to maintain cyclin destruction until entry into the next cell cycle
- Cdks activity must be prevented after mitosis to provide a stable G1 state in which growth and other factors can control entry into the next cycle



▼ APC antagonistic function by ubiquitination **of S and M cyclins**

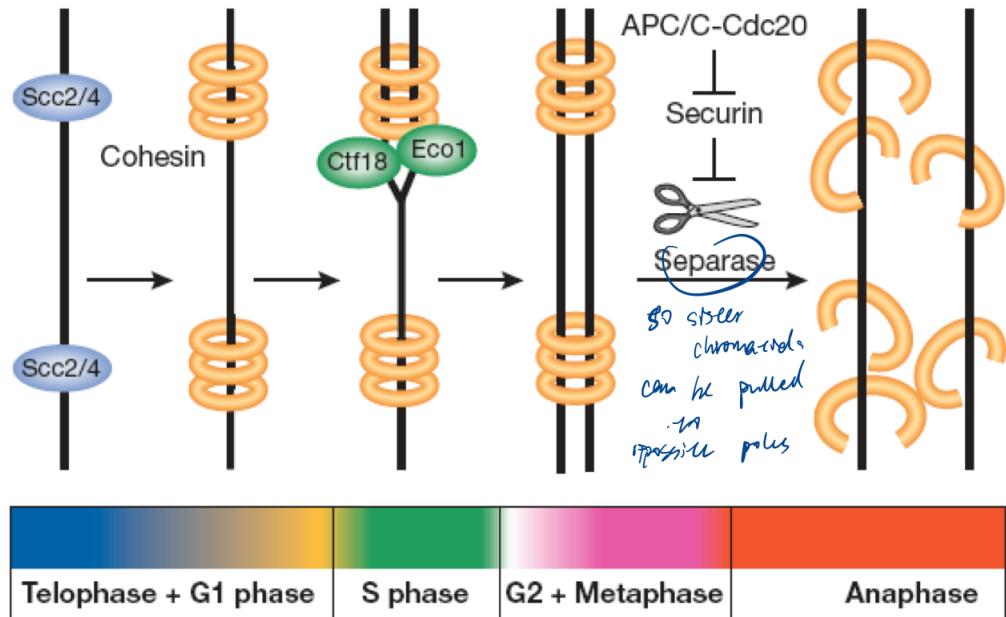
- M-Cdks **activate** APC by **phosphorylating** APC core subunits to enhance Cdc20 binding
- the degradation **reduces the activity of S- and M- Cdks** → **complete mitosis and cytokinesis**
- **This -ve loop ensures that APC activation and M-cyclins destruction only happen after M-Cdk activation** → **no premature destruction**



▼ APC promotes the transition into anaphase by **ubiquitination - of securin**

- M-Cdks activate APC by phosphorylating APC core subunits to enhance Cdc20 binding → ubiquitination of securin
- degradation of securin → separase activity is unleashed → destroys sister-chromatid **cohesion** → sister chromatids are drawn to opposite poles during metaphase

Cohesins physically link the sister chromatids

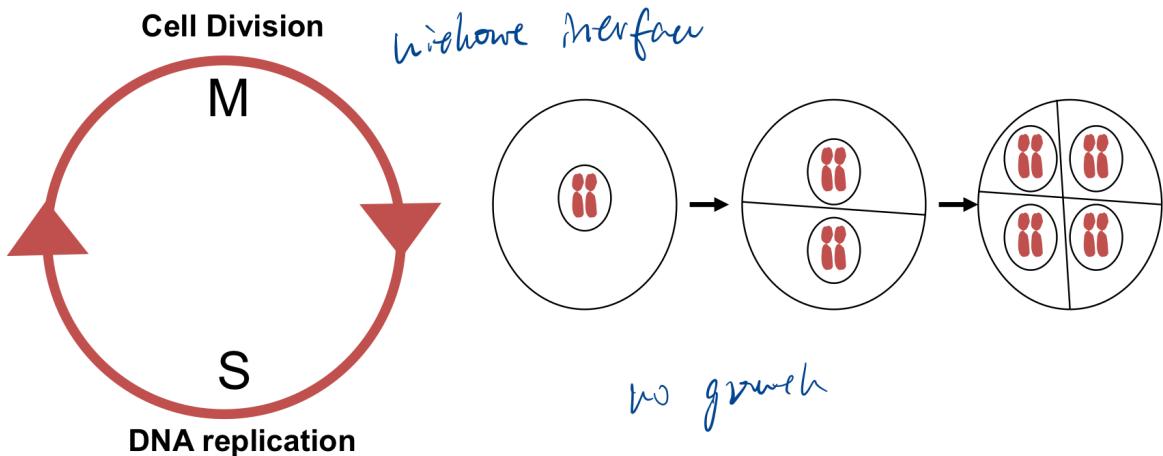


▼ APC Cdh1 adaptation

- M-Cdk activity decrease downregulates the phosphorylation of APC → Cdc20 dissociates → APC activity decreases → **at the end of mitosis APC Cdc20 is no longer active**

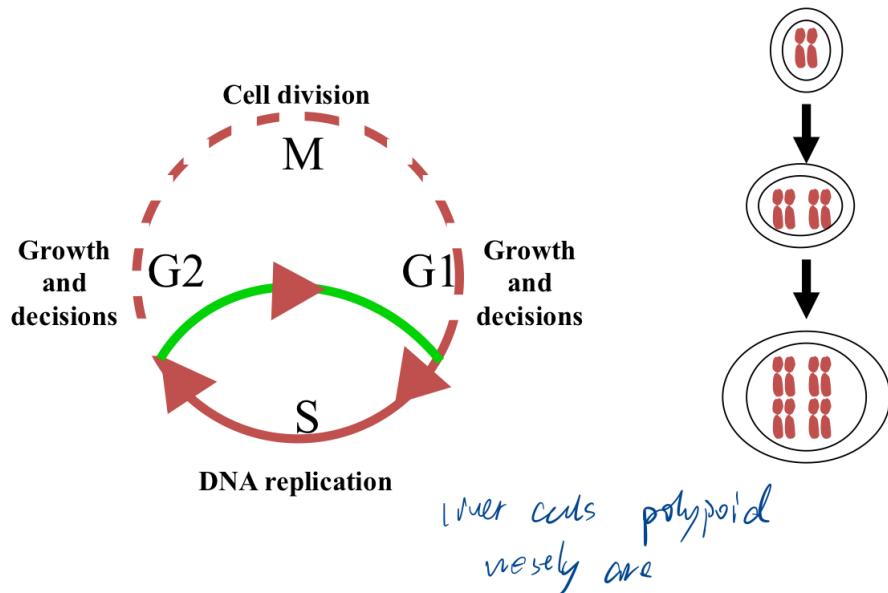
Modifications of the standard cell cycle

- Early embryonic cell does not have interface — no cell growth, but have continuous division



Simplified cell cycle (basic oscillator)

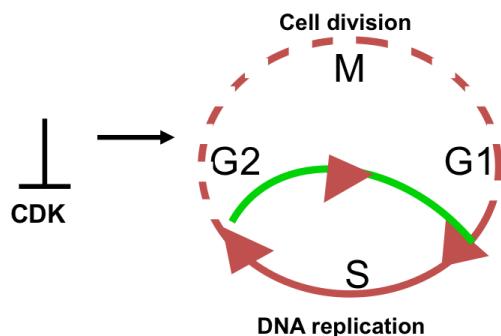
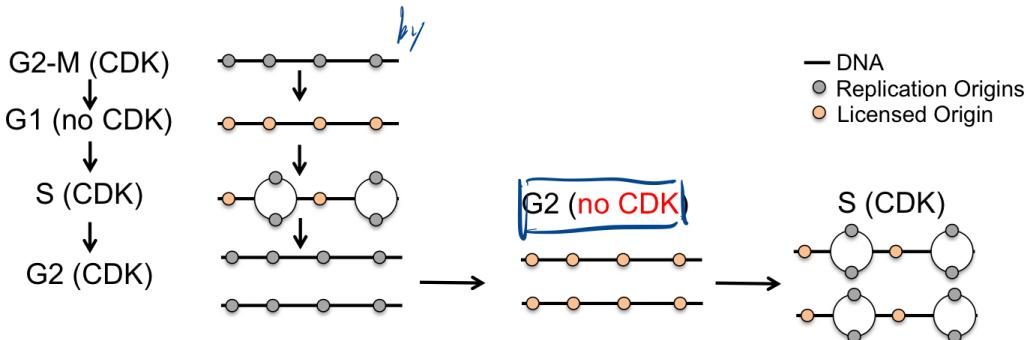
- Megakaryocytes: inhibition of M phase - large multinucleated cells



Megakaryocytes, where inhibition of M results in a large multinucleated cell

bypass prevention of biduplication

- This can be thought of after G2 phase, CDK activity is inhibited — reoccurrence of licensing — new round of S phase — endoreplication



Endoreduplication

Genetic experiments in yeast showed that inhibition of G2 cyclin/CDK led to endoreduplication (multiple rounds of S phase).

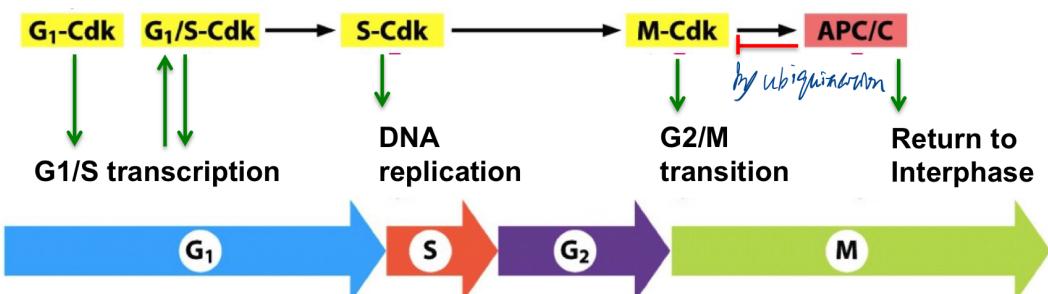
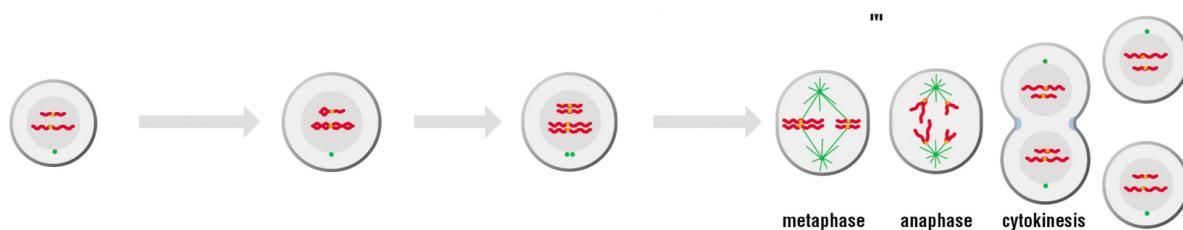


Figure 17-21 Molecular Biology of the Cell 5/e (© Garland Science 2008)



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Checkpoints

In other organisms than yeast:

1. G_1/S -cyclin activate Cdk in late G_1 and thereby help trigger progression through Start, resulting in a commitment to cell-cycle entry. Their levels fall in S phase.
2. S-cyclins bind Cdk soon after progression through Start and help stimulate chromosome duplication. S-cyclin levels remain elevated until mitosis, and these cyclins also contribute to the control of some early mitotic events.
3. M-cyclin activate Cdk that stimulate entry into mitosis at the G_2/M transition. M-cyclin levels fall in mid-mitosis.

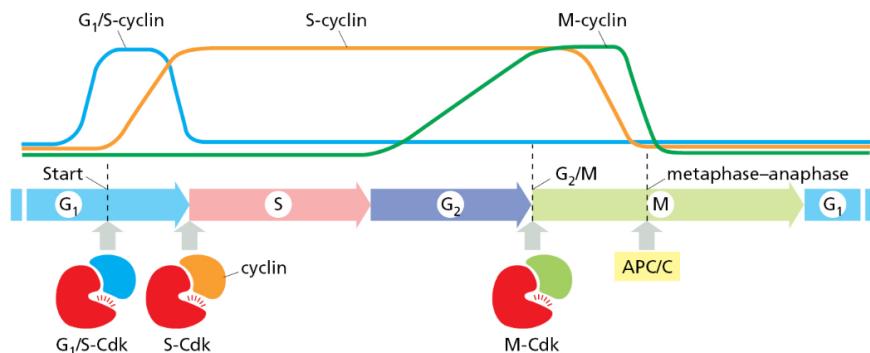
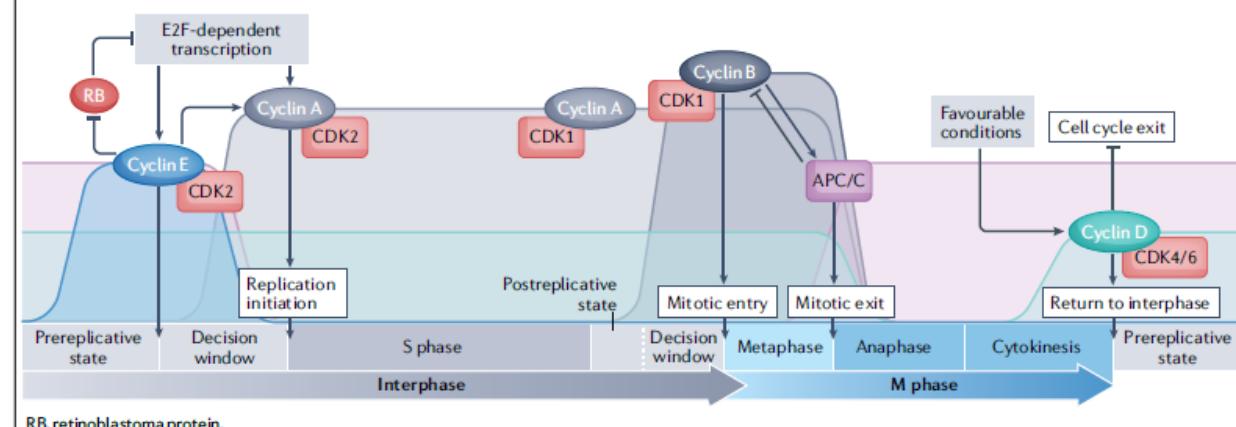


Figure 17–10 Cyclin–Cdk complexes of the cell-cycle control system. The concentrations of the three major cyclin types oscillate during the cell cycle, while the concentrations of Cdk (not shown) exceed cyclin amounts and do not change. In late G_1 , rising G_1/S -cyclin levels lead to the formation of G_1/S -Cdk complexes that trigger progression through the Start transition. S-Cdk complexes form later in G_1 and trigger DNA replication, as well as some early mitotic events. M-Cdk complexes form during G_2 but are held in an inactive state; they are activated at the end of G_2 and trigger entry into mitosis at the G_2/M transition. A separate regulatory protein complex, the APC/C, initiates the metaphase-to-anaphase transition, as we discuss later.

Cell cycle progression is driven by the accumulation of cyclin-dependent kinase (CDK) activity during interphase and M phase (see the figure). Loss of this activity, through APC/C in complex with the activator protein CDC20 (APC/C^{CDC20}) during mitosis, and then with CDH1 (APC/C^{CDH1}) during G1 leads to the degradation of cyclins, marking the return back to interphase. Cyclin D-CDK4/6 accumulation allows entry into the cell cycle, thereby preventing cell cycle exit. E2F-dependent transcription results in the accumulation of both cyclin E and cyclin A, which creates a decision window to enter S phase. Cyclin E-CDK2 activity further activates E2F-dependent transcription, creating a positive feedback loop

that results in increased cyclin E-CDK2 activity and cyclin A-CDK2 complex. This process allows the accumulation of cyclin A-CDK2 activity, through the inactivation of APC/C^{CDH1} activity (not shown), and replication initiation and S phase entry. Subsequent accumulation of cyclin A/B-CDK1 complex creates a second decision window, following S phase completion, for mitotic entry. Accumulation of cyclin A/B-CDK1 activity drives mitotic entry and allows APC/C^{CDC20} activation, which is required for mitotic exit and targeted degradation of cyclins, to complete a cell cycle. Under favourable conditions, accumulation of cyclin D-CDK4/6 activity allows cells to re-enter the cell cycle.

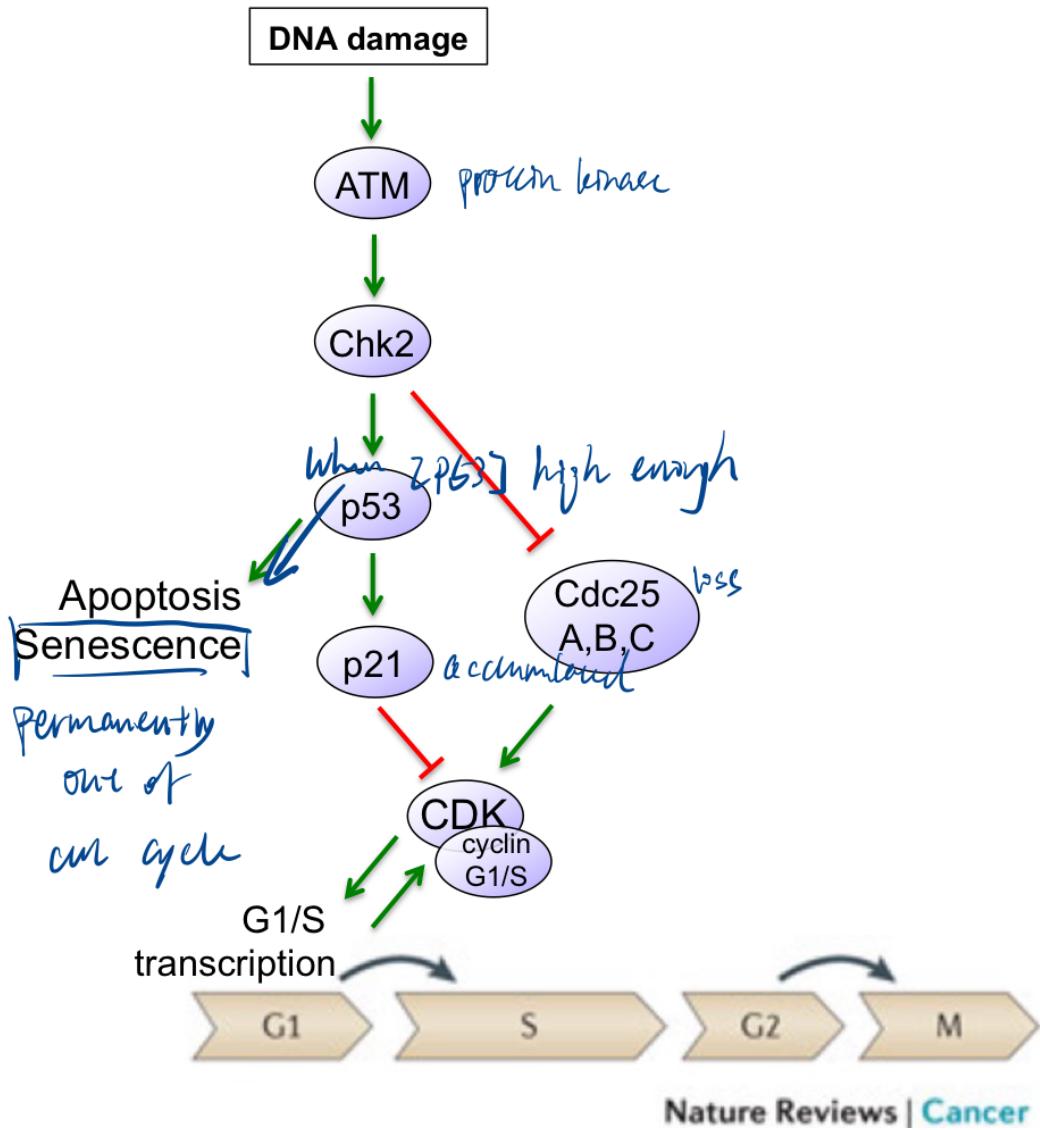


- G1-Cdk: cyclin D-Cdk complex
 - decision window whether the cell can commit to initiate replication

- the inhibition or absence of this activity leads the cell to quit the cell cycle and enter quiescence
- D-type cyclin level is accumulated when the **cell receives growth signals and mitogens** that stimulate largely the MAPK pathway
- Active G1-Cdk complex then causes inhibitory phosphorylation of RB (retinoblastoma protein), and thus activates the E2F-dependent transcription network
- This activation promotes the expression of E-type cyclins → activate CDK2 → **positive loop → inhibit RB → upregulate E2F-dependent transcriptional network**
- increased CyclinE-CDK2 complex & upregulated E2F-dependent transcriptional network → upregulated CyclinA → commit to replication
 - **The inhibition of RB activity is also driven by APC/C Cdh1 inhibitor**

Checkpoint 1: G1/S checkpoint - insufficient cell size, nutrients, DNA damage

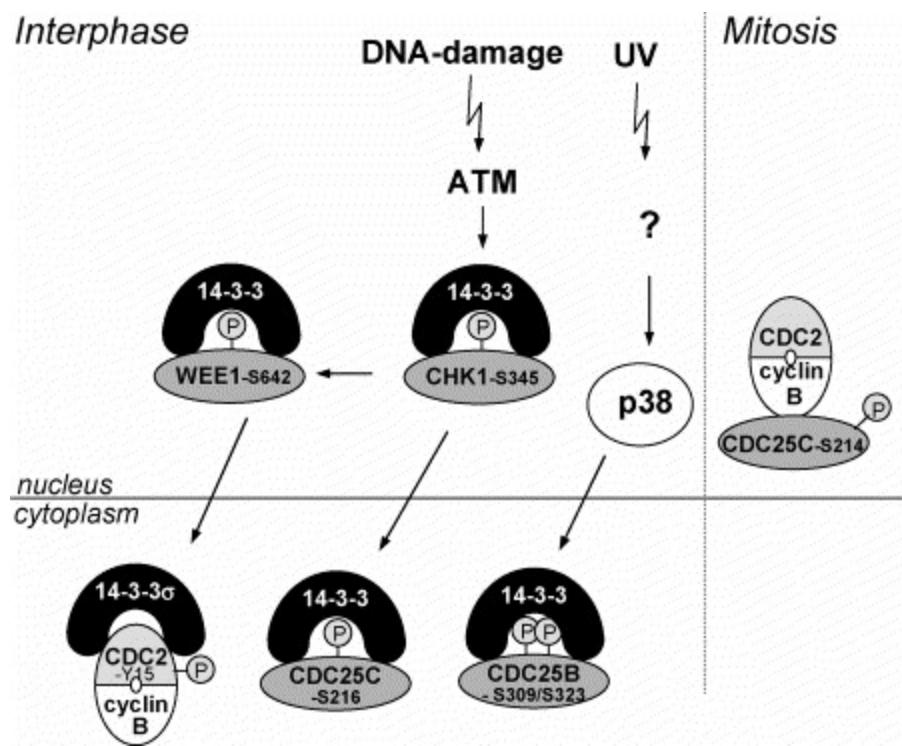
- If there is DNA double-strand breaks (DSB) → DNA damage sensor complex (MRN)
- MRN activates **ATM (ataxia telangiectasia mutated) → phosphorylates CHK2 (protein kinase) → p53 (transcription factor)**
- **p53 → p21 (CDK inhibitor) → downregulate G1-Cdk complexes & G1/S-Cdk → prevent S phase entry**
- **CHK2 during S and G2 phase: CHK2 degrades Cdc25 → inhibit M-Cdk activity → prevents entry of M phase**
- p53 and ATM are not critical during S and G2 phase
- p53 can also drive the cellular apoptosis and senescence → forcing the cell to get out of the cycle permanently



Checkpoint 2: DNA replication stress checkpoint

- functions only during S phase
- DNA replication stress: **the slowing or stalling of replication fork progression and/or DNA synthesis for faithful genome duplication**
 - This impediments can cause DNA replication forks to stall → **exposes single-stranded DNA (marker of replication stress) & decoupling of polymerase and helicase**

- single-stranded DNA → Activation of **ATR** → **CHK1** → Wee1
- Replication stress is not DNA damage; the purpose of the replication stress checkpoint is to **prevent replication stress-induced DNA damage & allow more time for replication to be completed**
- CHK1 phosphorylate Cdc25 → binding of 14-3-3 → inhibition & sequestration
- CHK1 phosphorylate Wee1 → activates activity



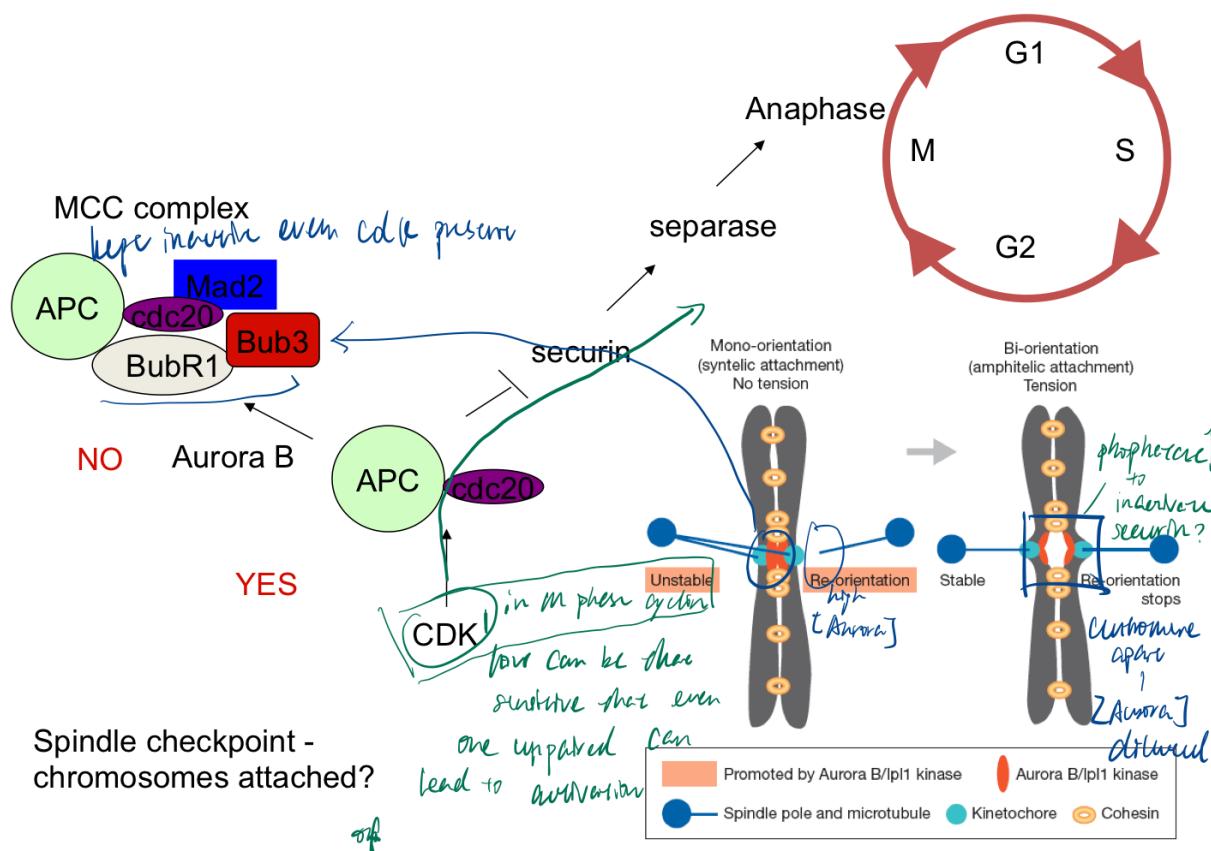
- Net result: downregulation of M-Cdk — CyclinB-Cdk1 — the cell does not enter M phase

Checkpoint 3: Spindle assembly checkpoint

- **During M phase:** To ensure that replicated DNA is partitioned equally between two daughter cells & prevent unattached or incorrectly attached kinetochores
 - errors can be: uncaptured chromosomes & two sister chromatids attached to the same spindle pole
- surveillance mechanism for unattached or incorrectly attached kinetochores

▼ multiprotein complex (SAC) that recruited to **any kinetochores not bound to microtubules following phosphorylation by Aurora B and CDK1**

- This phosphorylation enhances the formation of **MCC (mitotic checkpoint complex — MAD2, BUBR1, CDC20)** → act as **APC/cdc20 inhibitor** to inhibit anaphase initiation
- If the kinetochores are attached and bioriented: the checkpoint complex dissociates & free Cdc20 → Cdc20 now can act as a co-activator of APC/C
- Therefore, SAC can prolong the mitosis until bipolar spindle attachment is achieved by all chromosomes



- If the kinetochores of the sister chromatids are attached to different poles (**bi-orientation**) - the two are slightly pulled away at the **centromere region**:
 - Concentration of Aurora B (Kinase) is diluted out → Yes APC activation

- If the kinetochores are attached in mono-orientation to the same pole — the two centromere regions are tightly placed:
 - **Concentration of Aurora B is high → activate the formation of MCC complex**
- ▼ If the chromosome biorientation is not resolved following prolonged mitotic arrest:
 - caspase activation → apoptosis
 - slippage
 - Cyclin B degradation during prometaphase → **low CDK1 activity → low APC/Cdc20 & low CDK1**
 - The cells exit M phase without chromosome segregation
 - usually results in cell death or **cell cycle exit via p53**

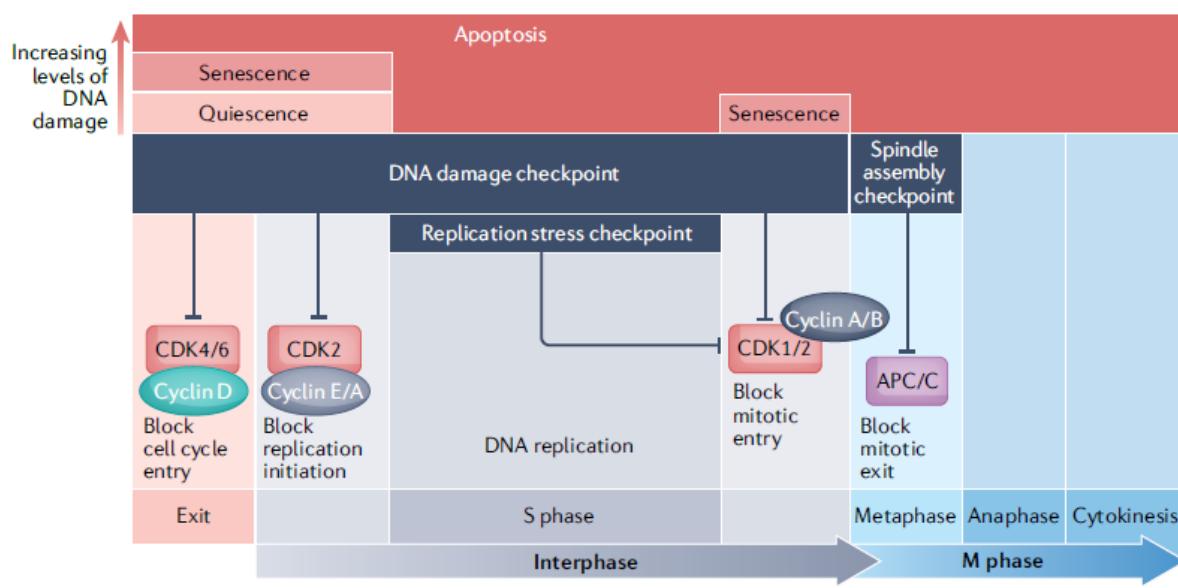


Fig. 2 | Checkpoint-dependent cell cycle arrest and exit. The replication stress checkpoint can block mitotic entry during S phase by preventing the accumulation of cyclin-dependent kinase 1/2 (CDK1/2)-cyclin A/B activity, and the spindle assembly checkpoint can block mitotic exit during M phase by preventing the activation of the anaphase-promoting complex/cyclosome (APC/C). By comparison, the DNA damage checkpoint operates throughout interphase. Depending on the phase of the cell cycle, the DNA damage checkpoint can either block mitotic entry during and following S phase, much like the replication stress checkpoint, or block replication initiation in pre-S phase by preventing the accumulation of cyclin E/A-CDK2 activity. It can also block cell cycle entry following mitotic exit, or during a prolonged pre-S phase, by preventing or inhibiting cyclin D-CDK4/6 activity, thereby inducing a reversible cell cycle exit known as quiescence. In response to high levels of DNA damage, the DNA damage checkpoint can induce an irreversible exit from the cell cycle, through senescence, outside S and M phases, or even cell death through apoptosis throughout the cell cycle.

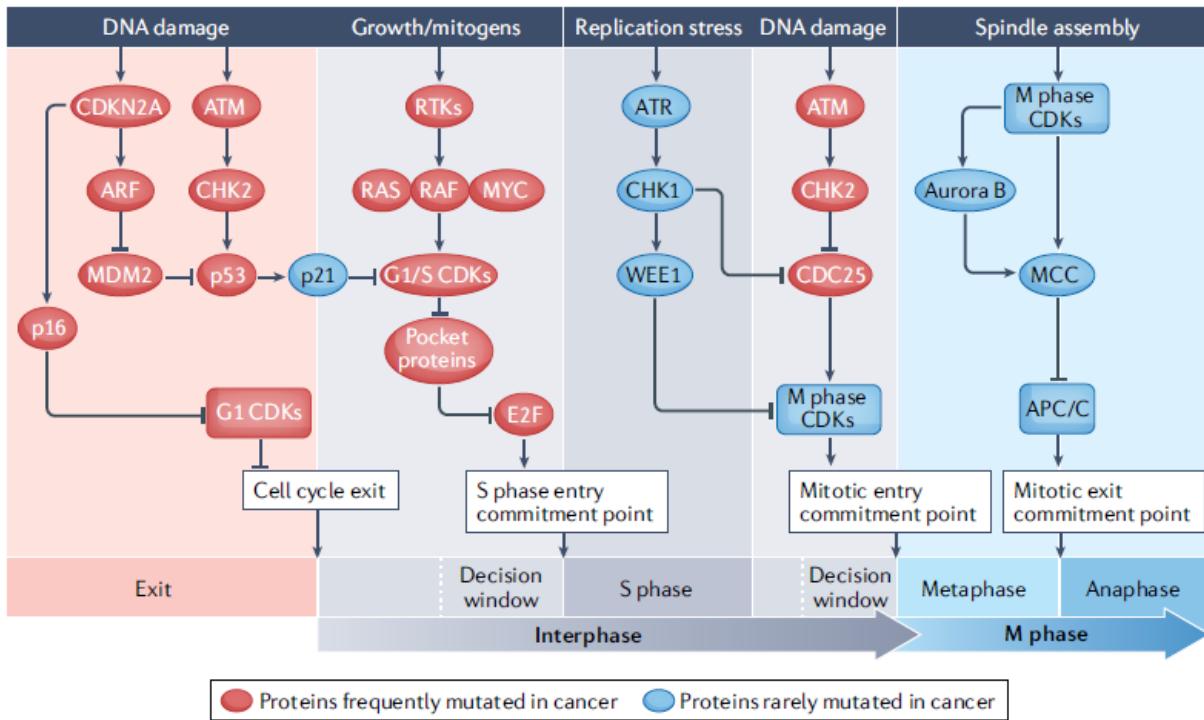


Fig. 3 | Key signalling pathways involved in cell cycle control and cancer. Continued cell cycle progression in cancer cells is driven mainly by mutations, or deregulation, of proteins involved in cell cycle-control signalling pathways. However, these mutations are associated with specific cell cycle control pathways more so than others. Mutations commonly found in cancer cells are shown in red: they affect mainly cell cycle control in response to DNA damage and growth signals in pre-S phase. These mutations drive S phase entry and prevent cell cycle exit. Very few cancer-associated mutations are found in proteins involved in the response to replication stress or incomplete spindle assembly; proteins that are rarely mutated in cancer are shown in blue. In the context of cancer treatment, these pathways represent therapeutic opportunities. Pocket proteins include retinoblastoma protein (RB), p107 and p130. E2F includes the activating E2Fs E2F1–E2F3 and indicates E2F-dependent transcription. G1/S cyclin-dependent kinases (CDKs) include cyclin D–CDK4/6 and cyclin E–CDK2. M phase CDKs include cyclin A/B–CDK1/2, APC/C, anaphase-promoting complex/cyclosome; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; MCC, mitotic checkpoint complex (BUB3 together with MAD2 and MAD3 bound to CDC20); RTKs, receptor tyrosine kinases.

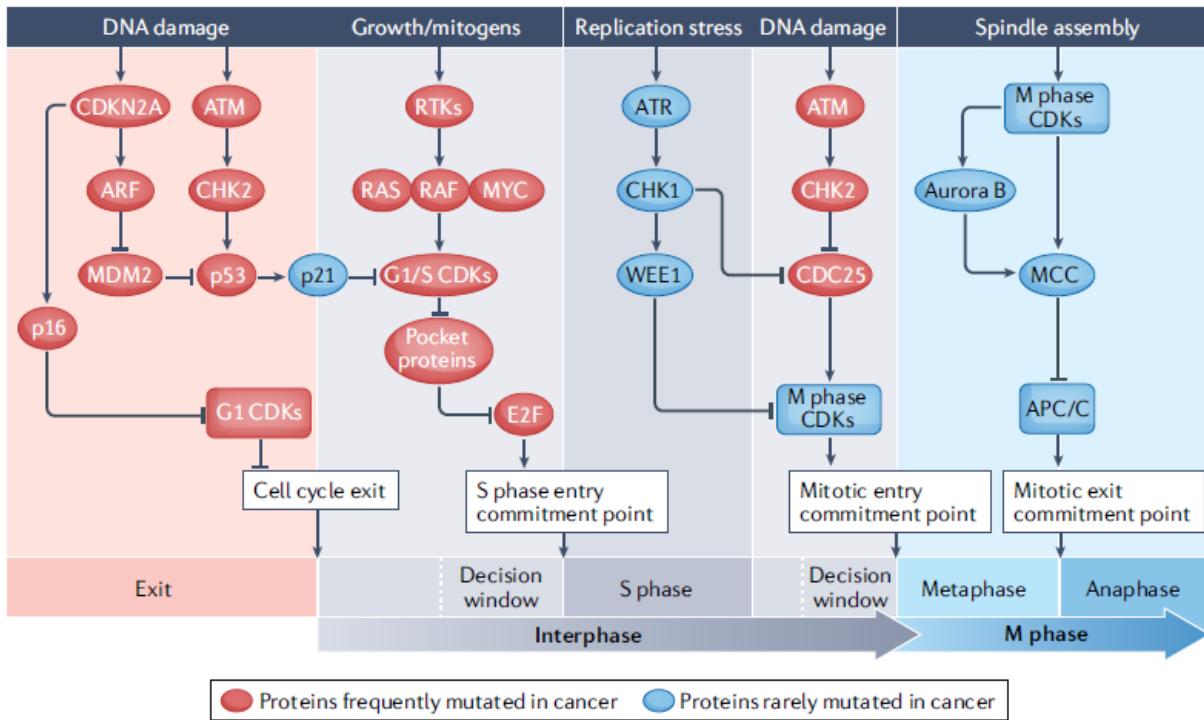
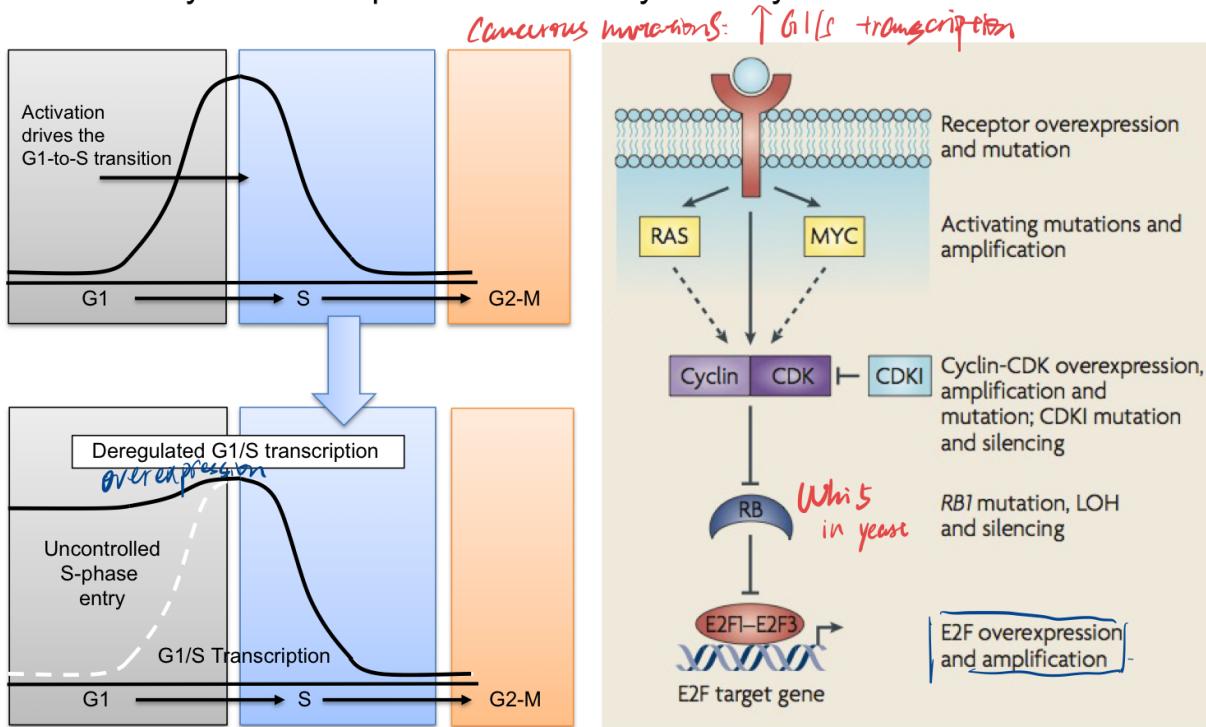


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Cell Cycle and Cancer

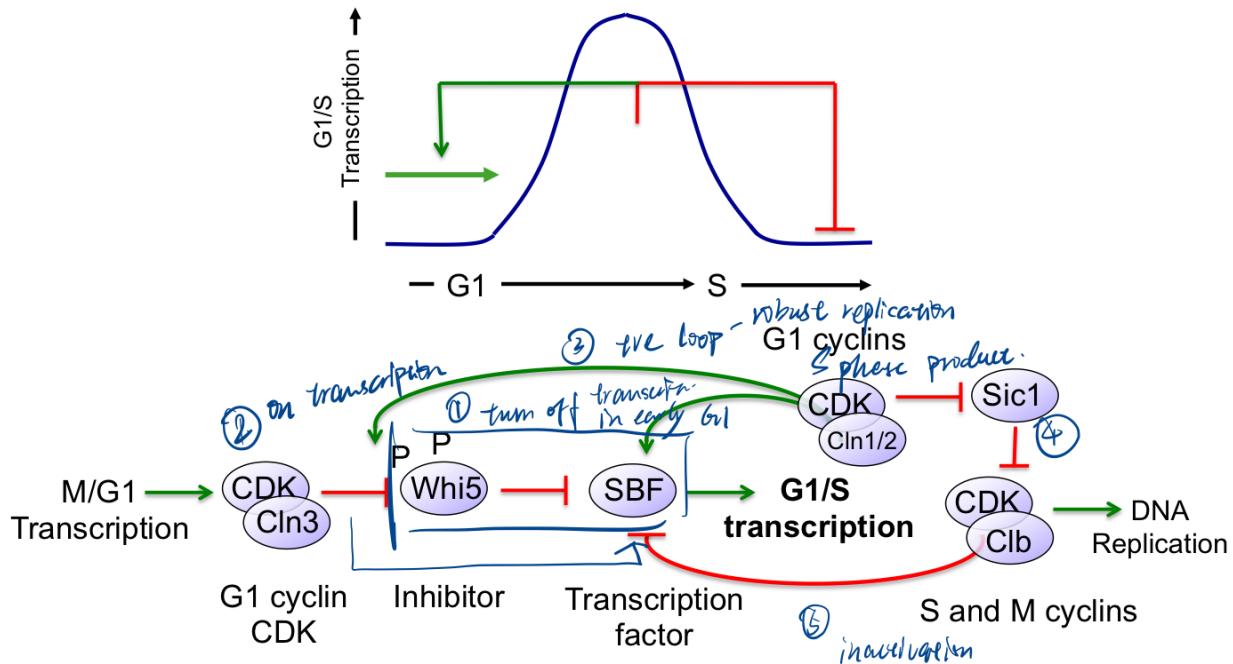
G1/S cell cycle transcription drives cell cycle entry



Adapted from: Chen, Tsai and Leone, Nature reviews, Cancer 2009

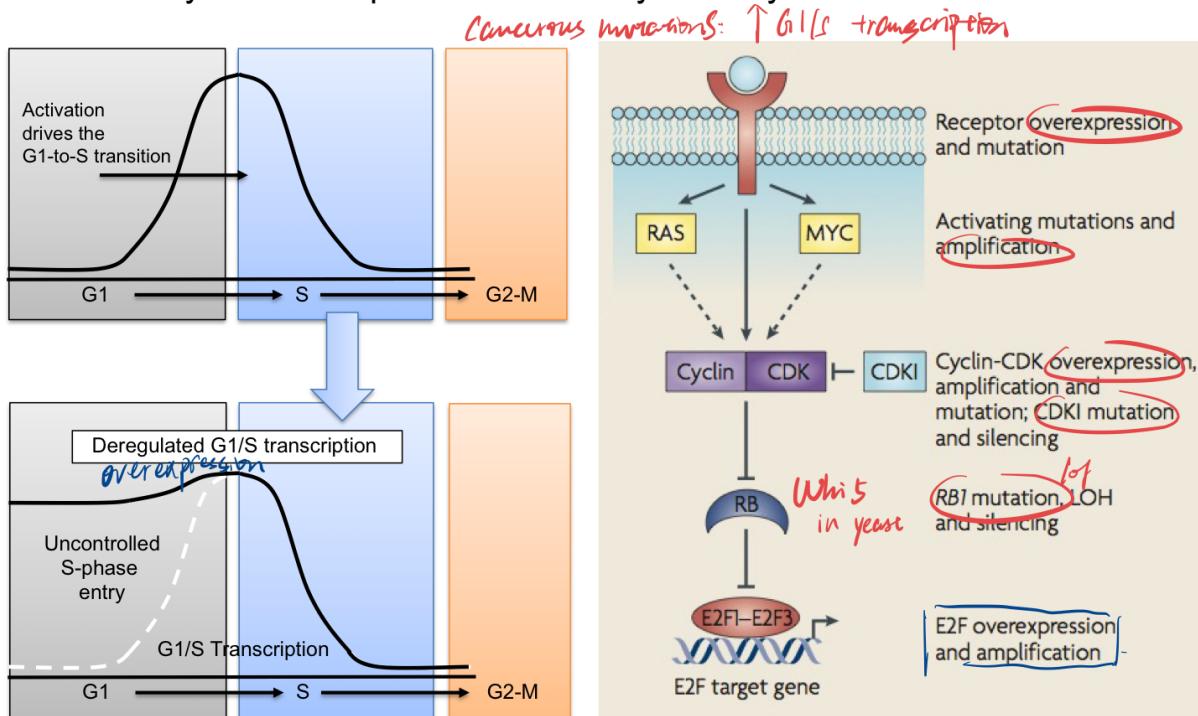
G1/S transcription initiates the G1 to S transition

Robust and committed activation linked to inactivation: Feedback!

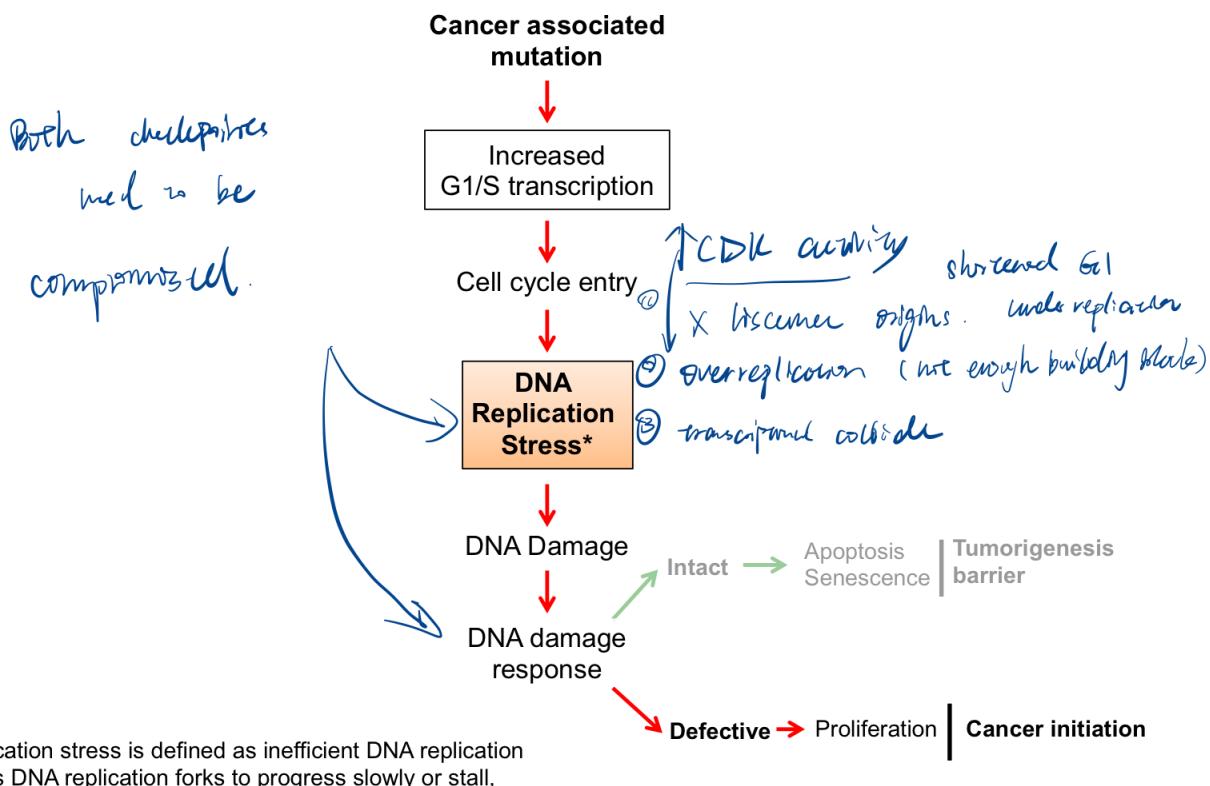


- Cell receives growth signals — increased G1-Cdk activity & APC/Cdh
- Whi5 (RB) initially inhibited SBF thus G1/S transcription
- CyclinE-Cdk inhibits RB — upregulation of E2F-dependent transcription — ↑ G1/S & S transcription (Cyclin A-Cdk2)

G1/S cell cycle transcription drives cell cycle entry



Adapted from: Chen, Tsai and Leone, Nature reviews, Cancer 2009



- cell enters S phase unprepared → replication stress → upregulate the chances of DNA damage
- → during replication there is DNA damage checkpoint → kick the cell out of the cell cycle
- → defective mutation can lead to aberrant cell cycle