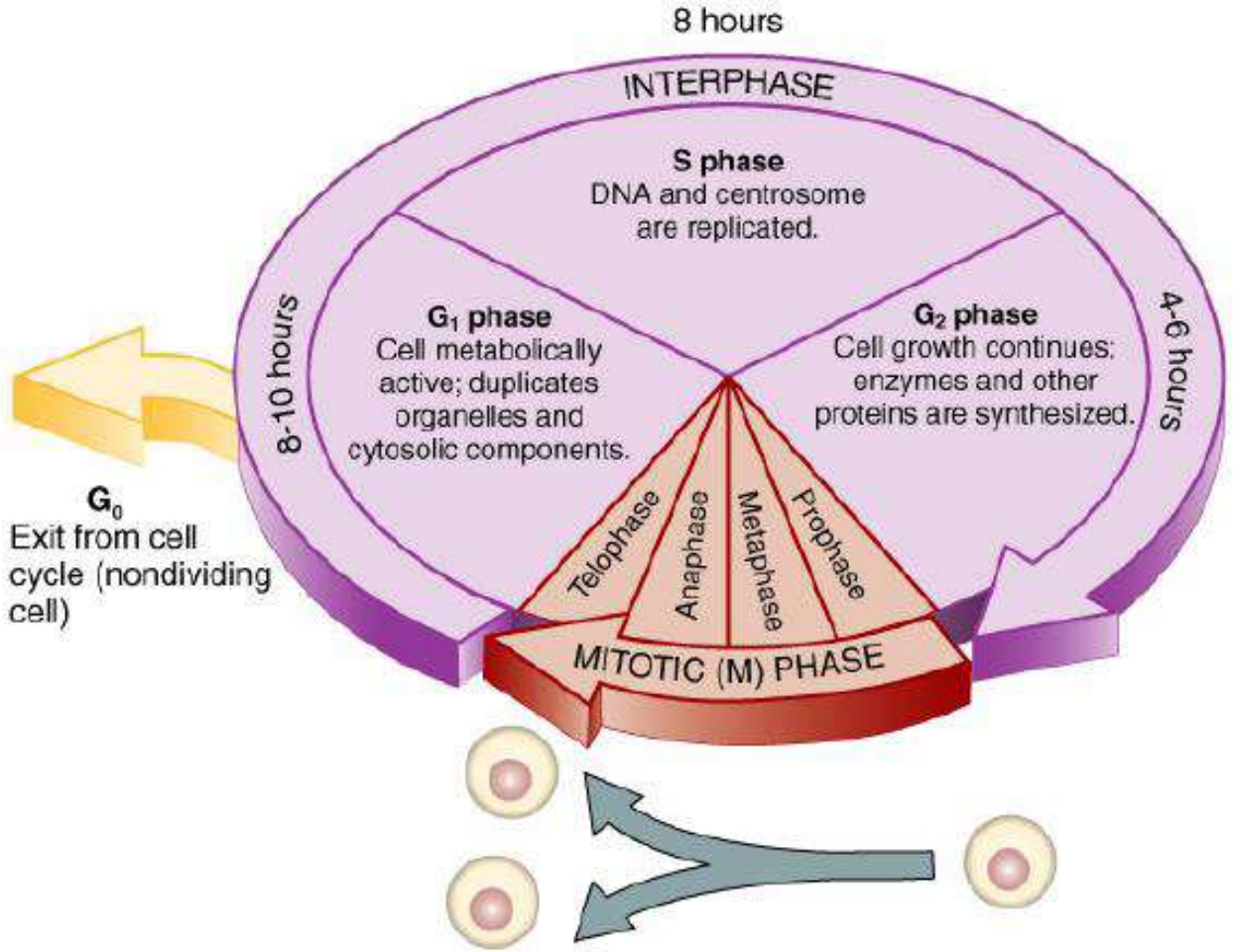
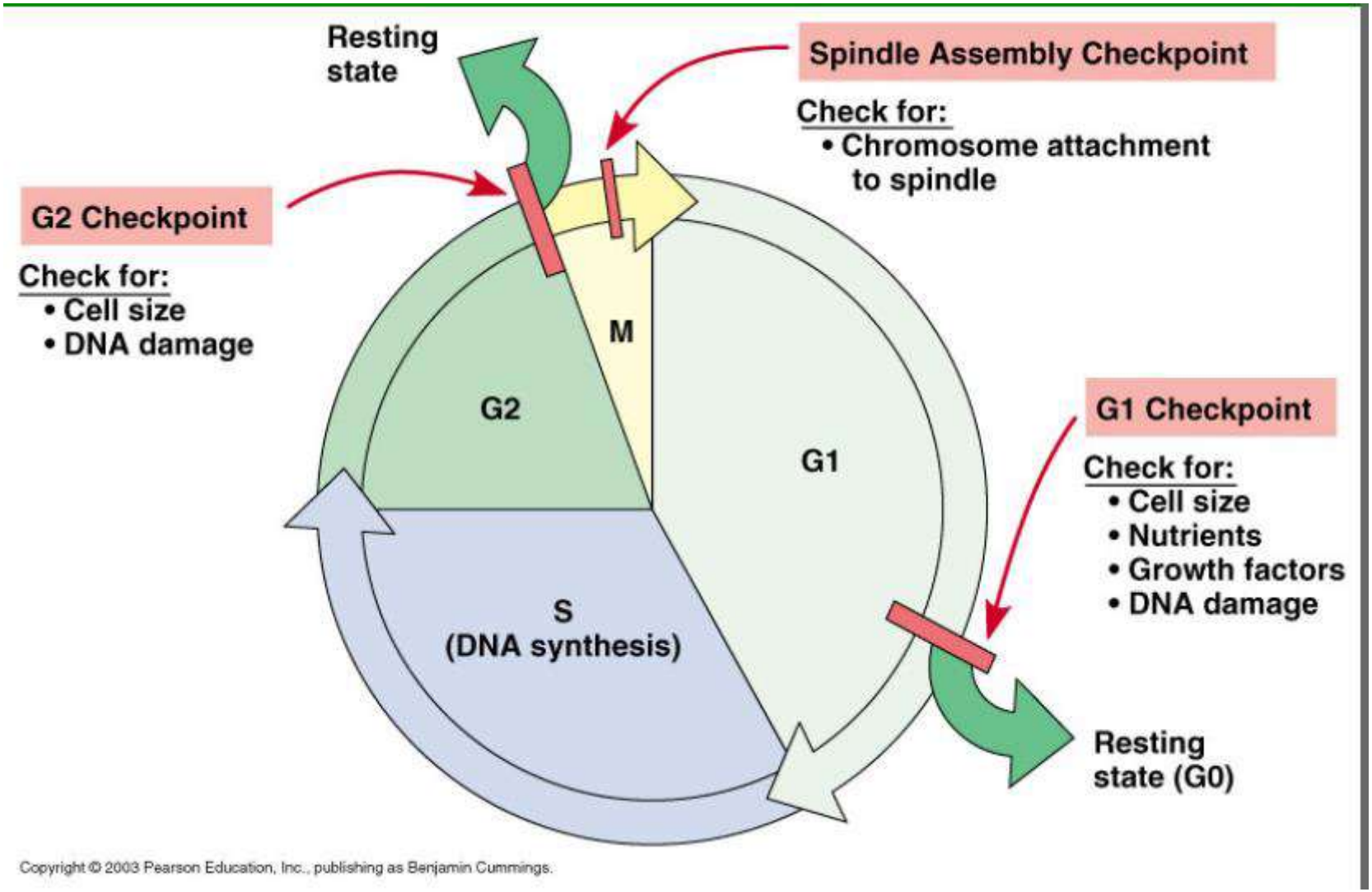
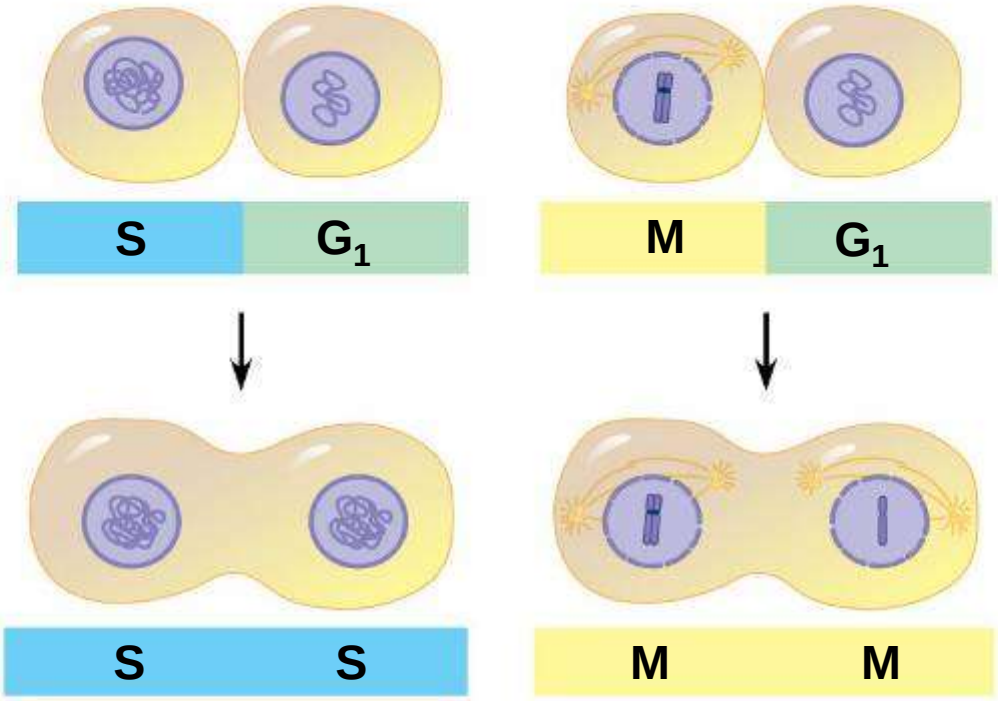
Cell Cycle



The cell cycle is a succession of very well organized, sophisticated, tightly controlled molecular events that give the ability to the cell to produce the exact itself's copy. Regulation of the cell cycle is critical for the normal development of multicellular organisms, and loss of control ultimately leads to cancer……..*fundamental cause*



G2 were fused to S-phase cells, G2 nuclei does not replicate.

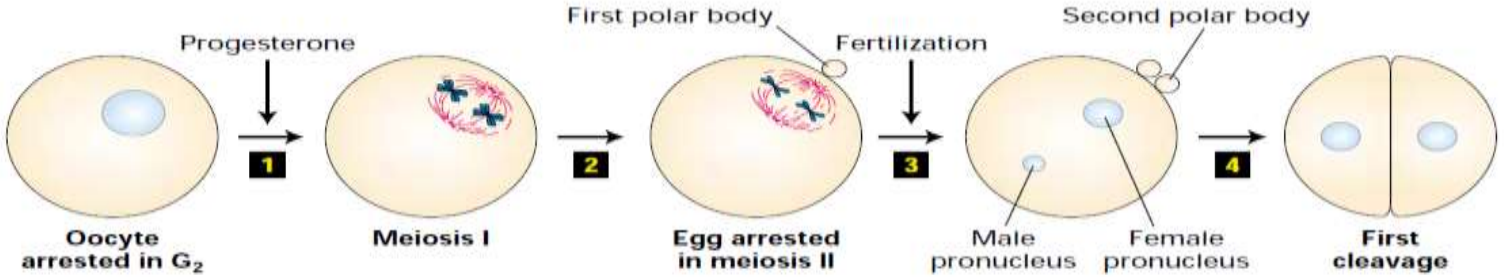


G_1 nucleus immediately entered the S phase—DNA was synthesized.

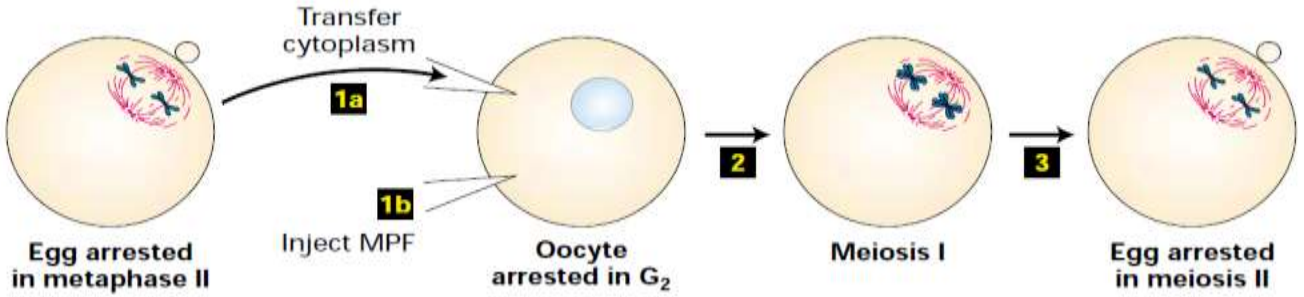
G1 nucleus immediately began mitosis

Thus diffusible
factors in an S-phase cell can enter the nucleus of a G1 cell and stimulate DNA synthesis, but these factors cannot induce DNA synthesis in a G2 nucleus

Maturation-Promoting Factor (MPF) discovery

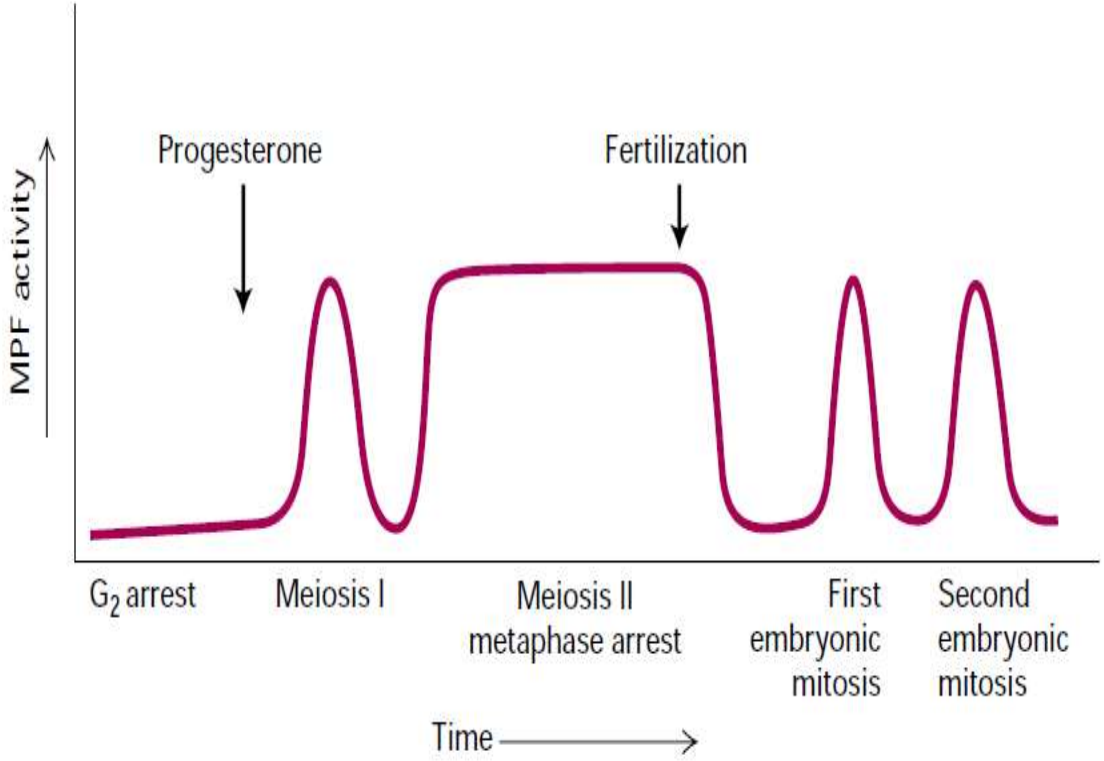


▲ EXPERIMENTAL FIGURE 21-5 Progesterone stimulates meiotic maturation of *Xenopus* oocytes in vitro. Step 1: Treatment of G_2-arrested *Xenopus* oocytes surgically removed from the ovary of an adult female with progesterone causes the oocytes to enter meiosis I. Two pairs of synapsed homologous chromosomes (blue) connected to mitotic spindle microtubules (red) are shown schematically to represent cells in metaphase of meiosis I. Step 2: Segregation of homologous chromosomes and a highly asymmetrical cell division expels half the chromosomes into a small cell called the first polar body. The oocyte immediately commences meiosis II and arrests in metaphase to yield an egg. Two chromosomes connected to spindle microtubules are shown schematically to represent egg cells arrested in metaphase of meiosis II. Step 3: Fertilization by sperm releases eggs from their metaphase arrest, allowing them to proceed through anaphase of meiosis II and undergo a second highly asymmetrical cell division that eliminates one chromatid of each chromosome in a second polar body. Step 4: The resulting haploid female pronucleus fuses with the haploid sperm pronucleus to produce a diploid zygote, which undergoes DNA replication and the first mitosis of 12 synchronous early embryonic cleavages.



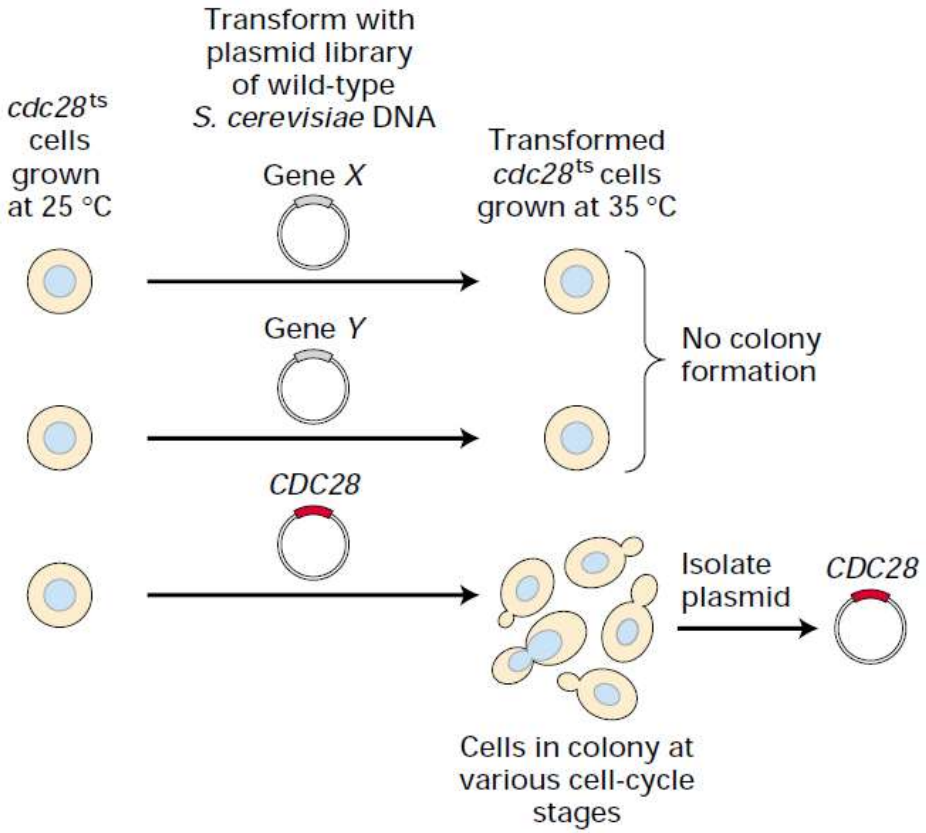
▲ EXPERIMENTAL FIGURE 21-6 A diffusible factor in arrested *Xenopus* eggs promotes meiotic maturation. When ≈5 percent of the cytoplasm from an unfertilized *Xenopus* egg arrested in metaphase of meiosis II is microinjected into a G_2-arrested oocyte (step 1a), the oocyte enters meiosis I (step 2) and proceeds to metaphase of meiosis II (step 3), generating a mature egg in the absence of progesterone. This process can be repeated multiple times without further addition of progesterone, showing that egg cytoplasm contains an oocyte maturation-promoting factor (MPF). Microinjection of G_2-arrested oocytes provided the first assay for MPF activity (step 1b) at different stages of the cell cycle and in different organisms. [See Y. Masui and C. L. Markert, 1971, *J. Exp. Zool.* **177**:129.]

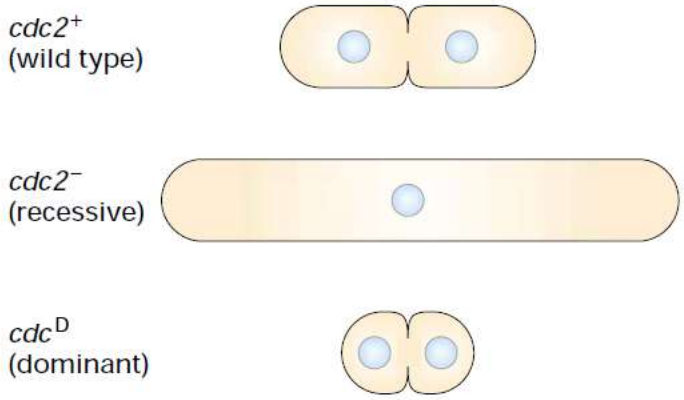
Cytoplasm from oocyte of 1st 12 h following progesterone treatment has no effect on recipient oocyte. Maximum activity after 20h of progestoren treatment.



◀ EXPERIMENTAL FIGURE 21-7 MPF activity in *Xenopus* oocytes, eggs, and early embryos peaks as cells enter meiosis and mitosis. Diagrams of the cell structures corresponding to each stage are shown in Figure 21-5. MPF activity was determined by the microinjection assay shown in Figure 21-6 and quantitated by making dilutions of cell extracts. See text for discussion. [See J. Gerhart et al., 1984, *J. Cell Biol.* **98**:1247; adapted from A. Murray and M. W. Kirschner, 1989, *Nature* **339**:275.]

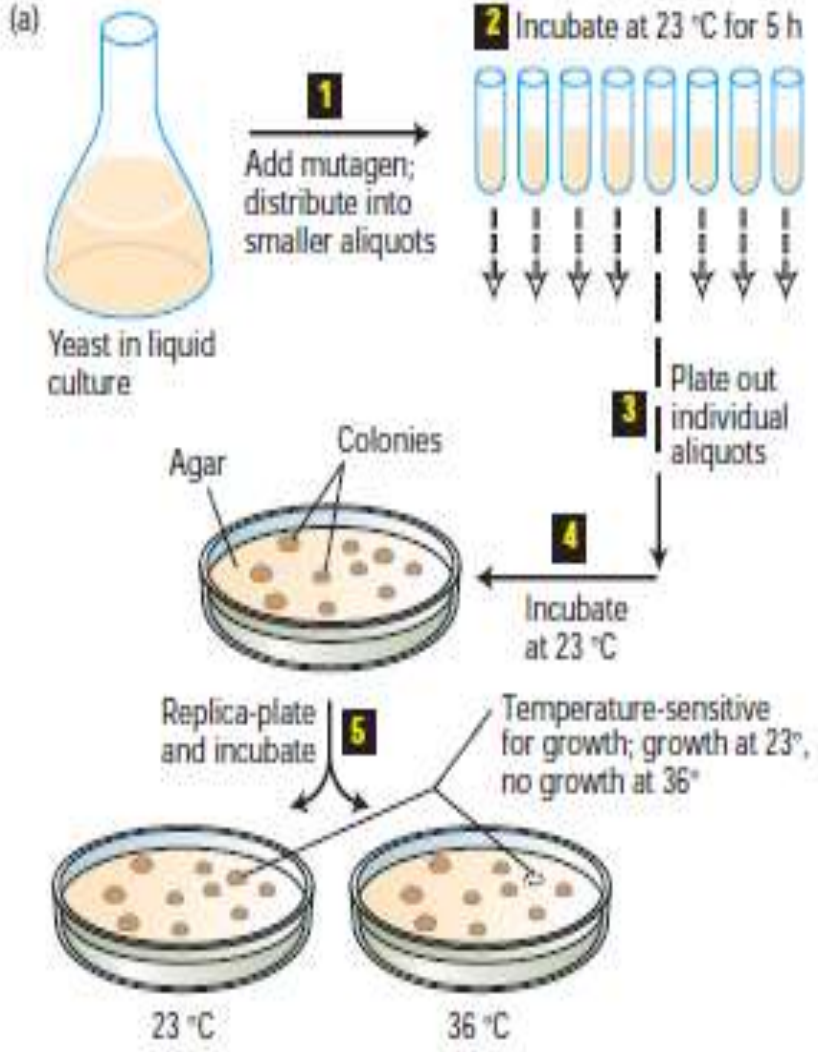
Strategy of Identification of cdc (cell division cycle) gene

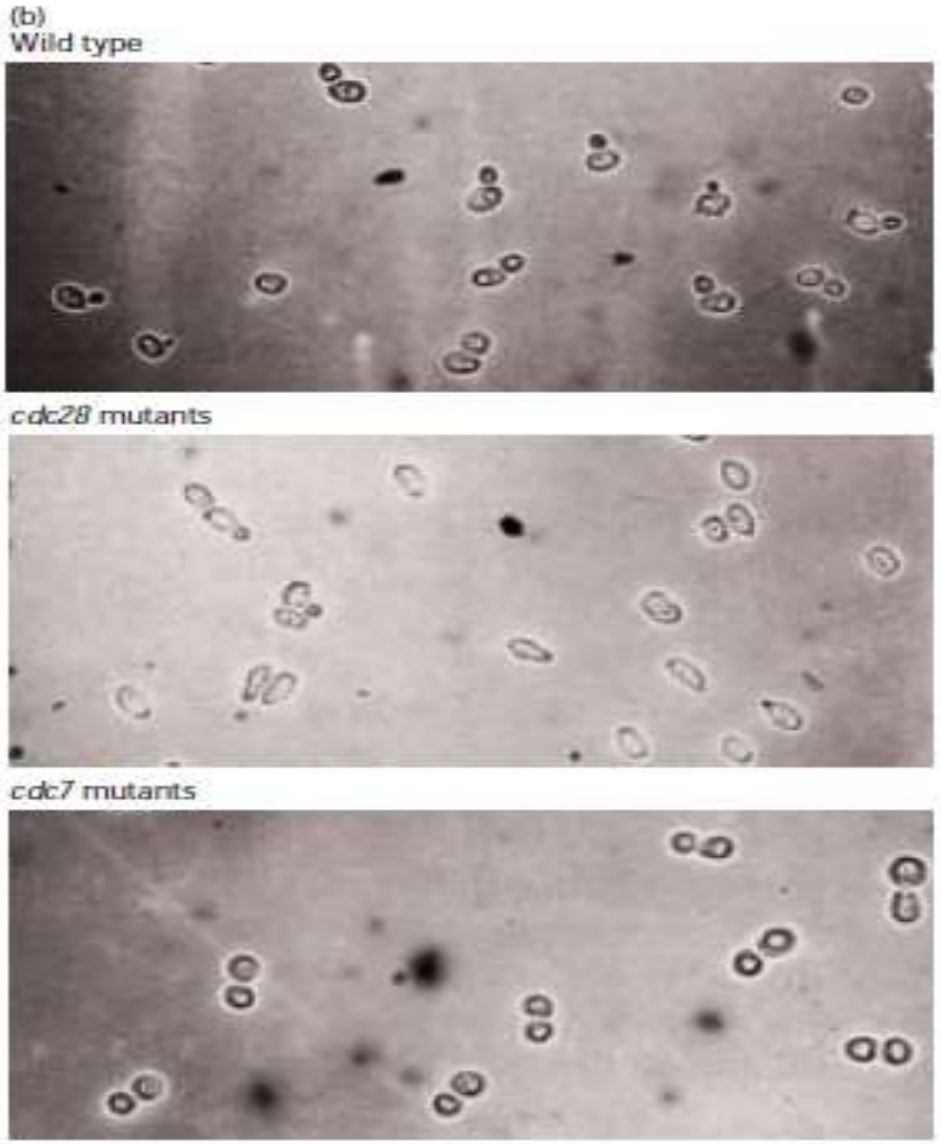


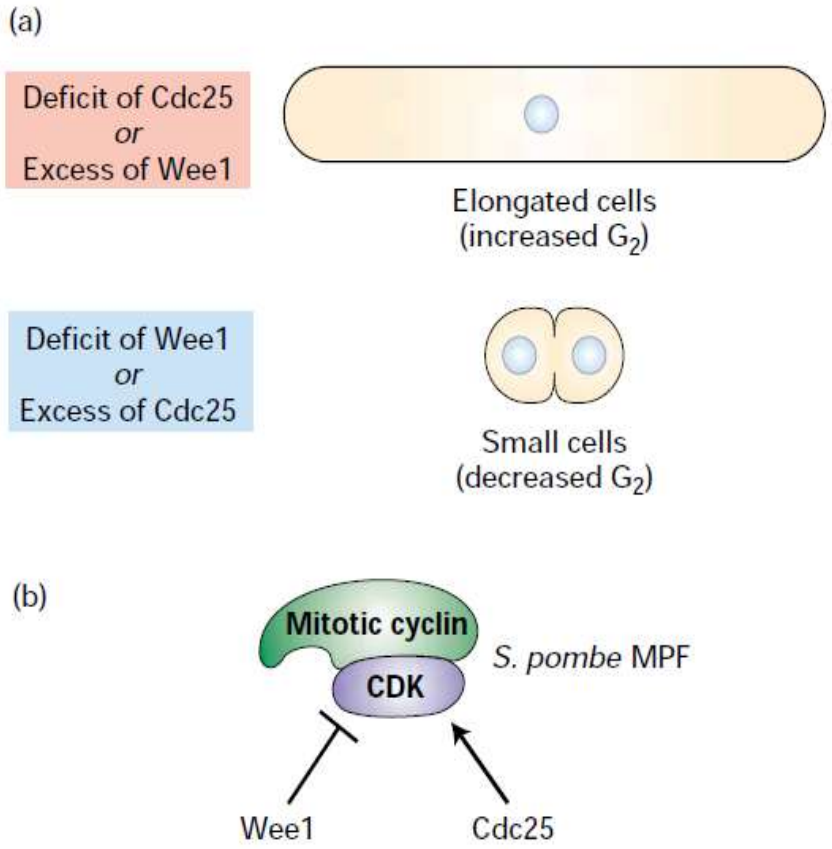


▲ **EXPERIMENTAL FIGURE 21-12 Recessive and dominant *S. pombe cdc2* mutants have opposite phenotypes.** Wild-type cell (*cdc2*$^{+}$) is schematically depicted just before cytokinesis with two normal-size daughter cells. A recessive *cdc2*$^{-}$ mutant cannot enter mitosis at the nonpermissive temperature and appears as an elongated cell with a single nucleus, which contains duplicated chromosomes. A dominant *cdc2*^{D} mutant enters mitosis prematurely before reaching normal size in G_2; thus, the two daughter cells resulting from cytokinesis are smaller than normal, that is, they have the wee phenotype.

Strategy of Identification of temperature sensitive mutant







temperature-sensitive *cdc25 mutants* are delayed in entering mitosis at the nonpermissive temperature, producing elongated cells.

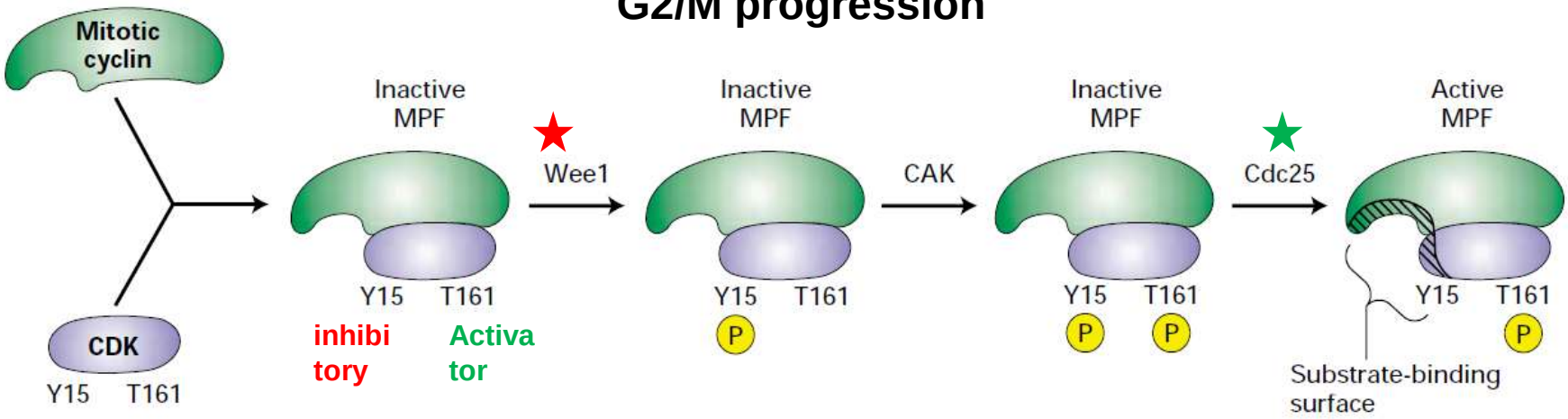
Over expression of Cdc25 decreases the length of G2 causing premature entry into mitosis and small (wee) cells.

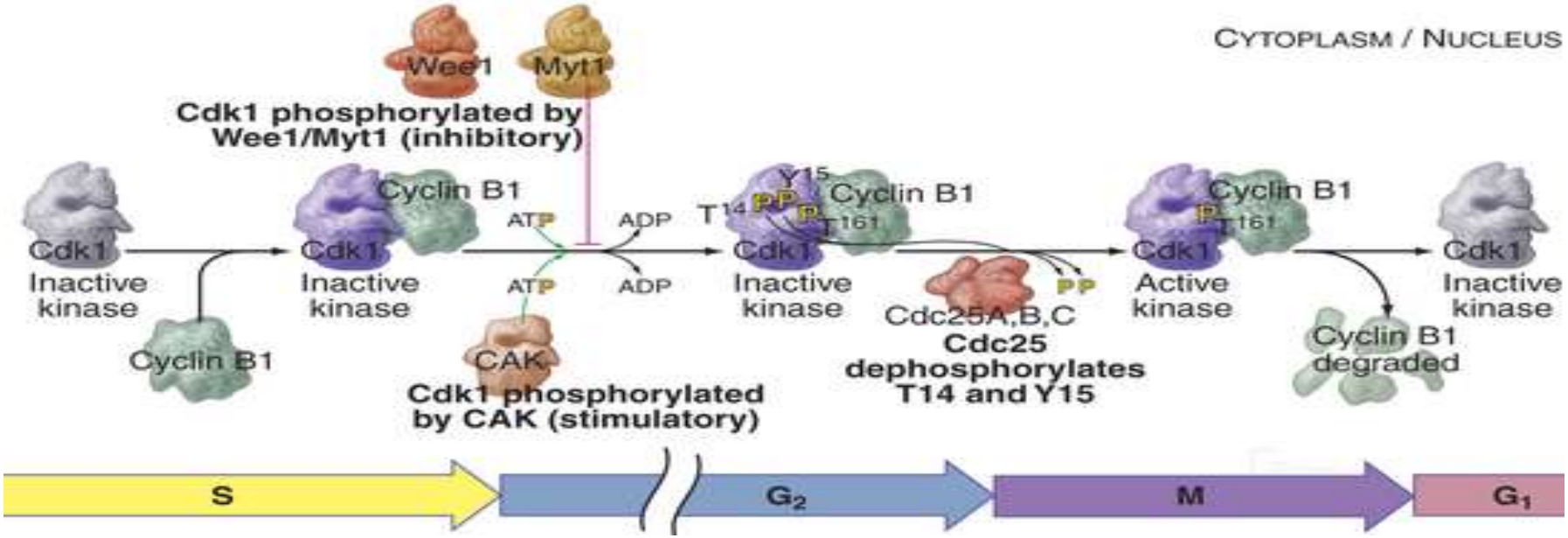
Conversely, loss-of-function mutations in the *wee1 gene causes* premature entry into mitosis resulting in small cells,

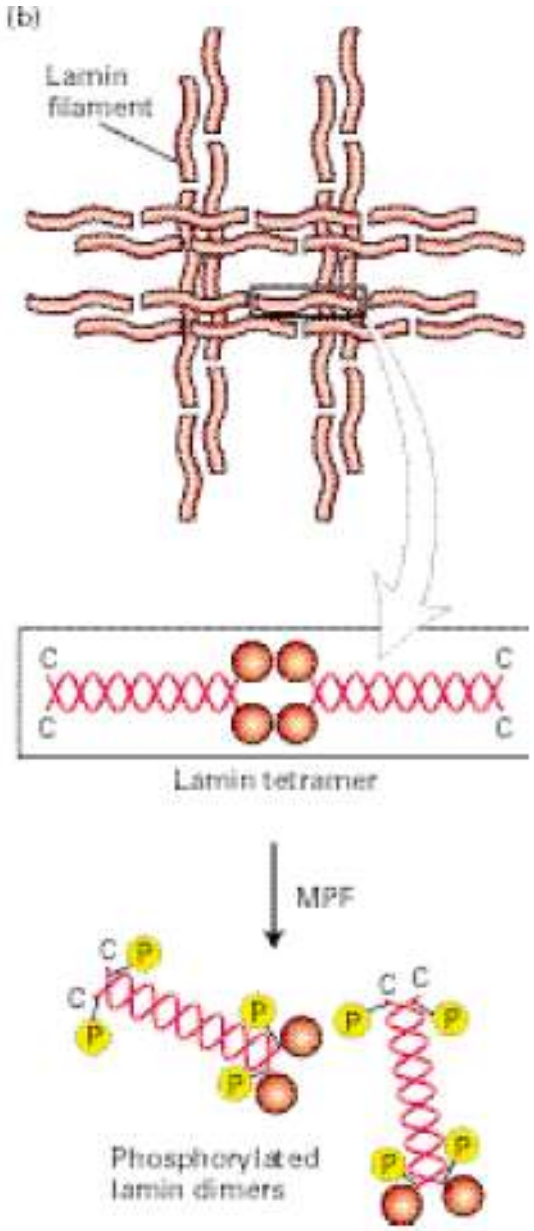
Whereas overproduction of Wee1 protein increases the length of G2 resulting in elongated cells.

Cdc25 protein stimulates the kinase activity of *S. pombe MPF, whereas Wee1 protein inhibits MPF activity*

G2/M progression

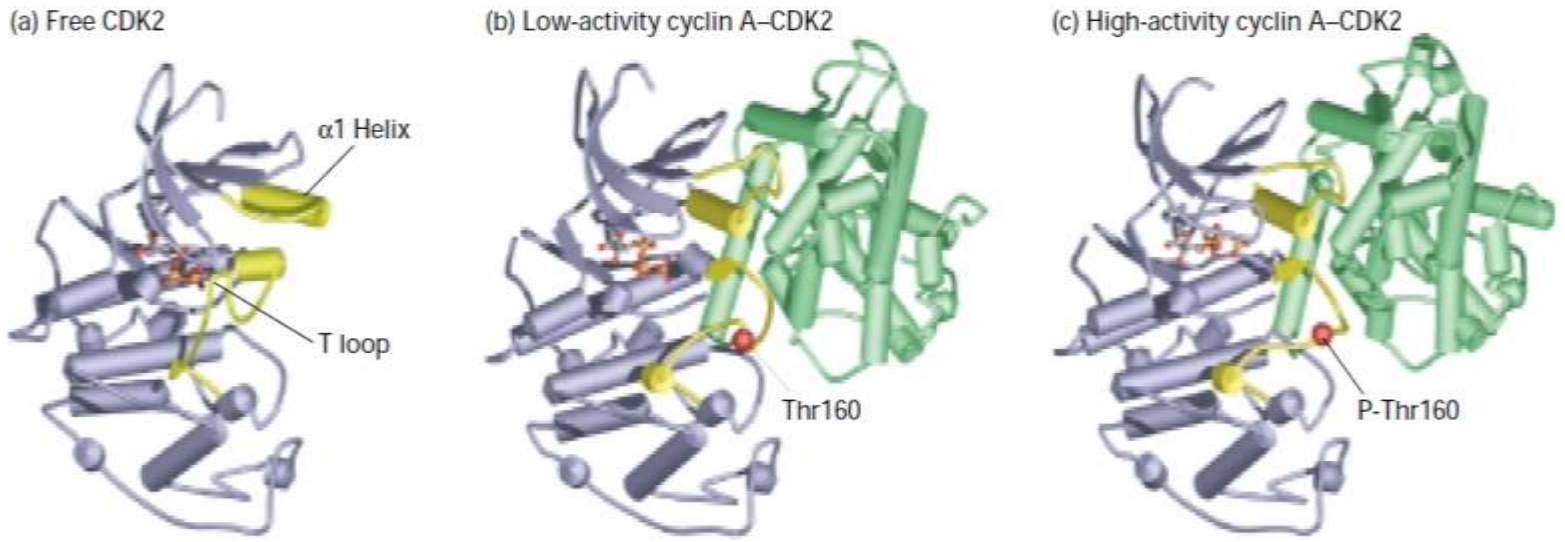




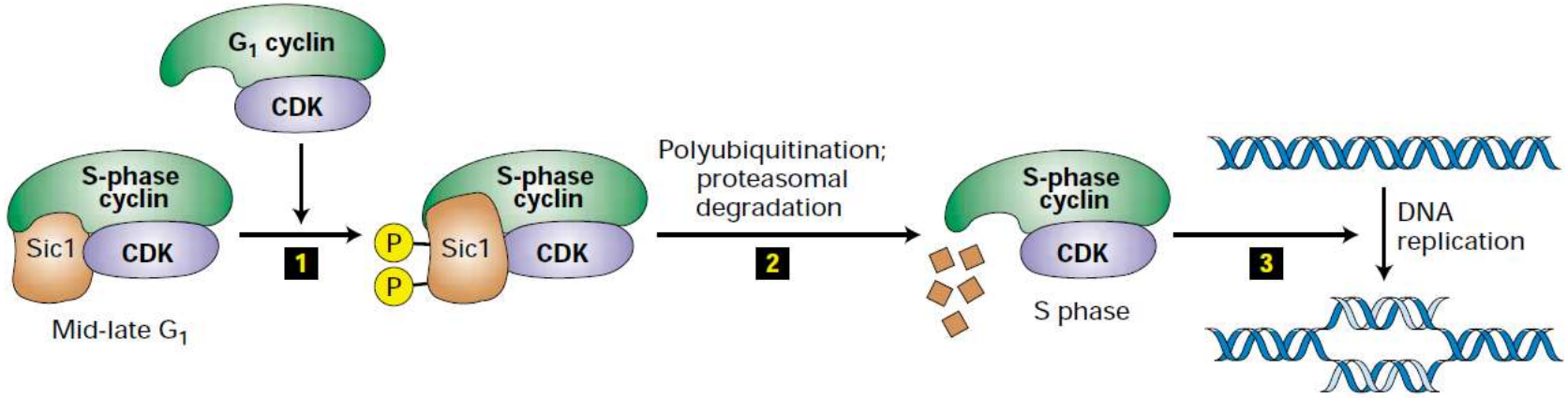


Double-membrane extension of the rough endoplasmic reticulum containing many nuclear pore. inner nuclear membrane is supported by the nuclear lamina, a meshwork of lamin filaments The three nuclear lamins (A, B, and C) present in vertebrate cells belong to the the intermediate filaments, Lamins A and C, encoded by alternative splicing of a single pre-mRNA, are identical except for a 133-residue regionnat the C-terminus of lamin A, which is absent in lamin C.

Lamin B, encoded by a different m RNA is modified post-transcriptionally by the addition of a hydrophobic isoprenyl group near its carboxyl-terminus. This fatty acid becomes embedded in the inner nuclear membrane, thereby anchoring the nuclear lamina to the membrane

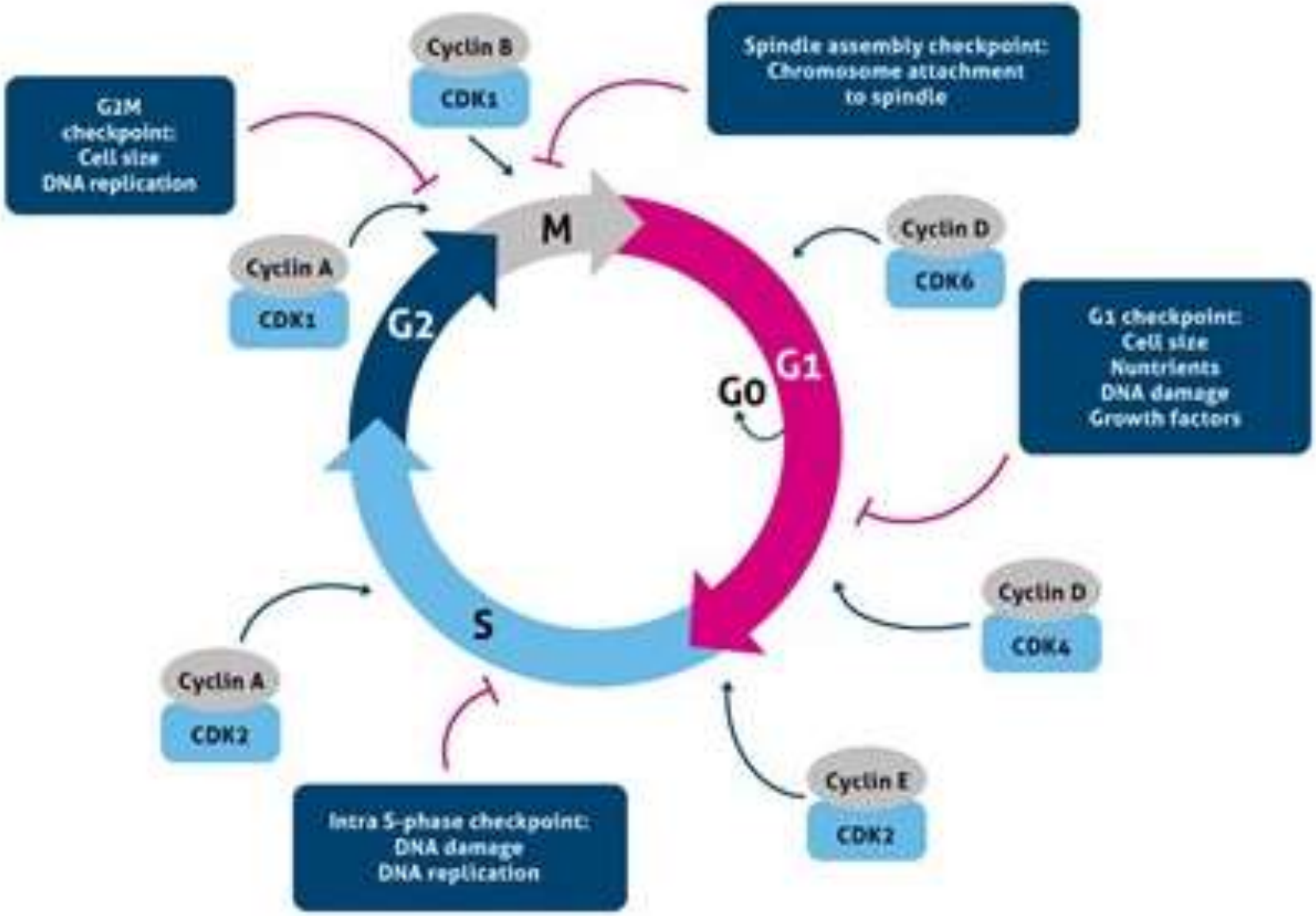


▲ FIGURE 21-15 Structural models of human CDK2, which is homologous to the *S. pombe* cyclin-dependent kinase (CDK). (a) Free, inactive CDK2 unbound to cyclin A. In free CDK2, the T-loop blocks access of protein substrates to the γ-phosphate of the bound ATP, shown as a ball-and-stick model. The conformations of the regions highlighted in yellow are altered when CDK is bound to cyclin A. (b) Unphosphorylated, low-activity cyclin A–CDK2 complex. Conformational changes induced by binding of a domain of cyclin A (green) cause the T-loop to pull away from the active site of CDK2, so that substrate proteins can bind. The α1 helix in CDK2, which interacts extensively with cyclin A, moves several angstroms into the catalytic cleft, repositioning key catalytic side chains required for the phosphorotransfer reaction to substrate specificity. The red ball marks the position equivalent to threonine 161 in *S. pombe* Cdc2. (c) Phosphorylated, high-activity cyclin A–CDK2 complex. The conformational changes induced by phosphorylation of the activating threonine (red ball) alter the shape of the substrate-binding surface, greatly increasing the affinity for protein substrates. [Courtesy of P. D. Jeffrey. See A. A. Russo et al., 1996, *Nature Struct. Biol.* **3**:696.]



▲ **FIGURE 21-24 Control of the $G_1 \rightarrow$ S phase transition in *S. cerevisiae* by regulated proteolysis of the S-phase inhibitor Sic1.** The S-phase cyclin-CDK complexes (Clb5-CDK and Clb6-CDK) begin to accumulate in G_1, but are inhibited by Sic1. This inhibition prevents initiation of DNA replication until the cell is fully prepared. G_1 cyclin-CDK complexes assembled in late G_1 (Cln1-CDK and Cln2-CDK) phosphorylate Sic1 (step 1), marking it for polyubiquitination by the SCF ubiquitin ligase, and subsequent proteasomal degradation (step 2). The active S-phase cyclin-CDK complexes then trigger initiation of DNA synthesis (step 3) by phosphorylating substrates that remain to be identified. [Adapted from R. W. King et al., 1996, *Science* **274**:1652.]

clb 5, clb 6 - S phase cyclin
cln 1, cln 2 - late G1 cyclin


Cyclin B
CDK1
Spindle assembly checkpoint:
Chromosome attachment
to spindle
G2M
checkpoint:
Cell size
DNA replication
M
Cyclin A
CDK1
G2
Cyclin D
CDK6
G1 checkpoint:
Cell size
Nutrients
DNA damage
Growth factors
G1
G0
S
Cyclin D
CDK4
Cyclin A
CDK2
Cyclin E
CDK2
Intra S-phase checkpoint:
DNA damage
DNA replication

The Retinoblastoma protein controls the G1/S transition

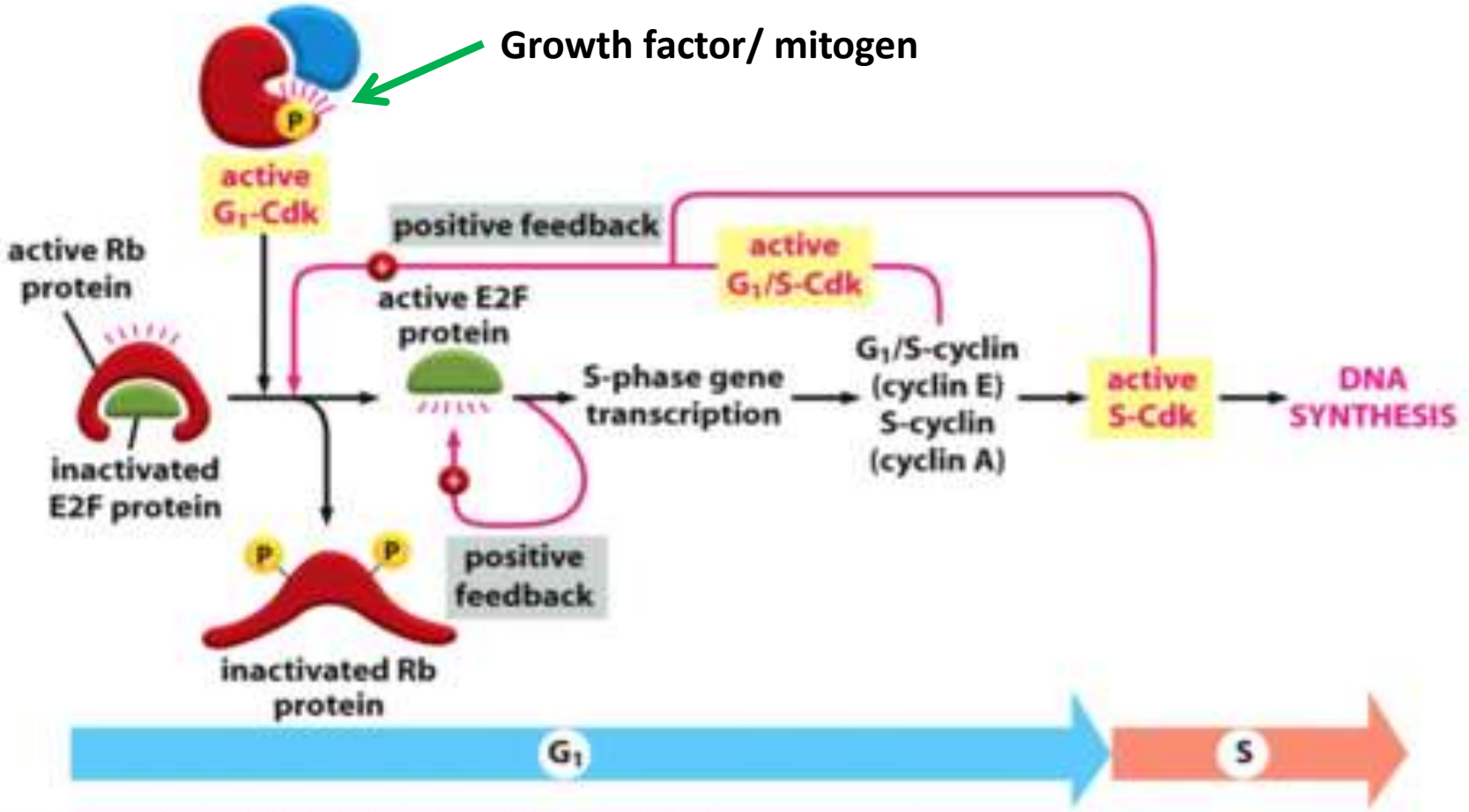
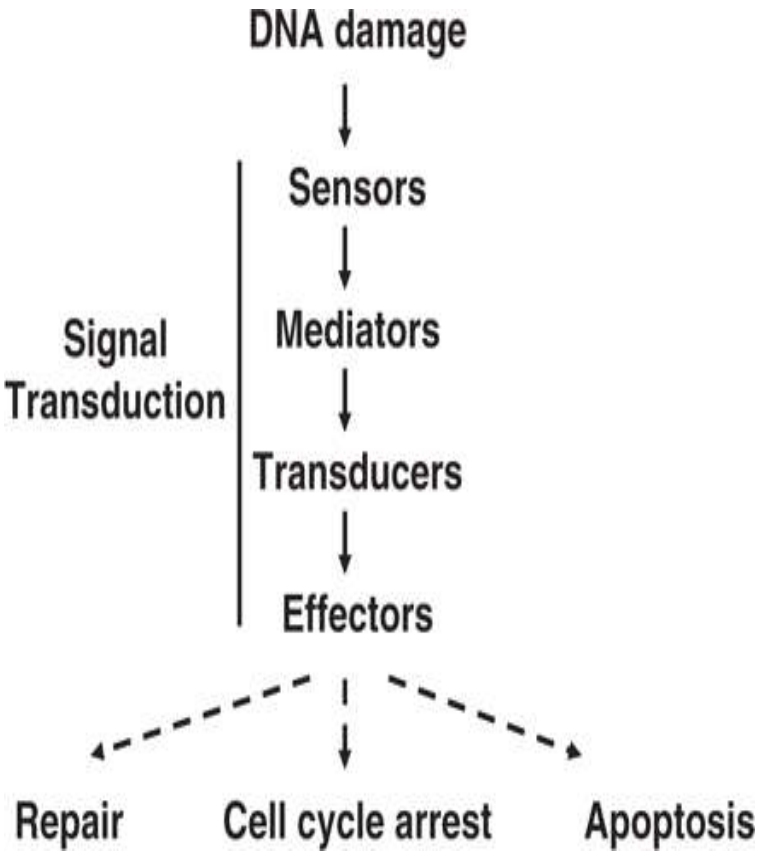
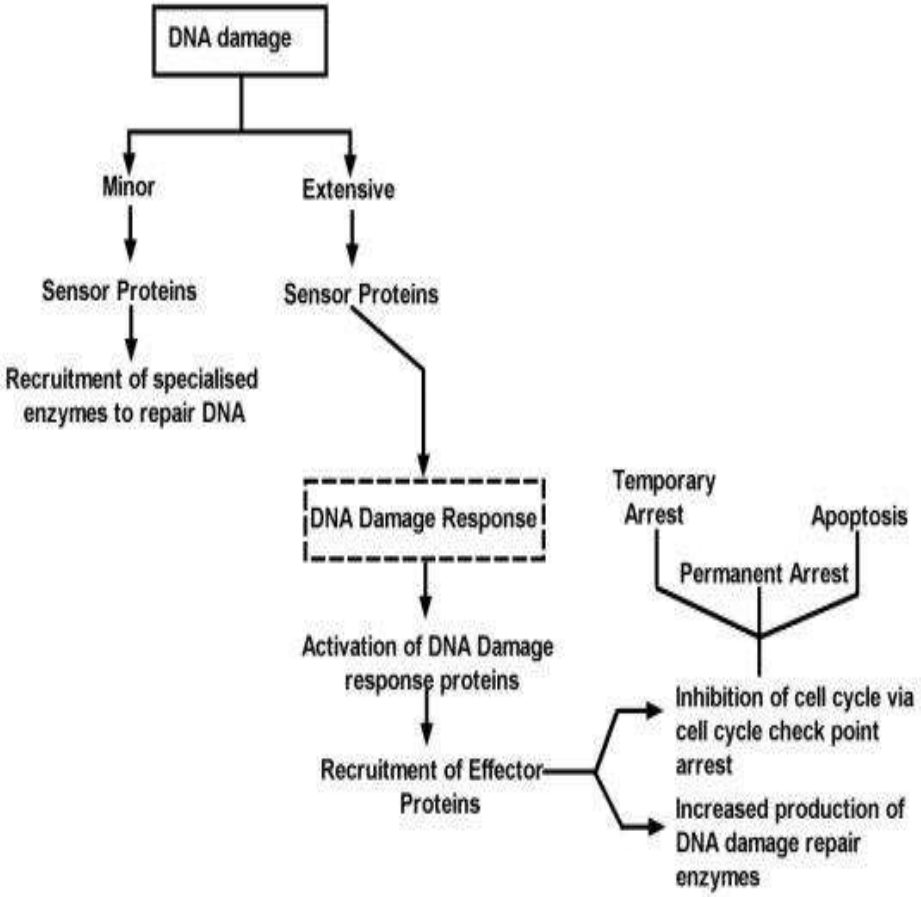


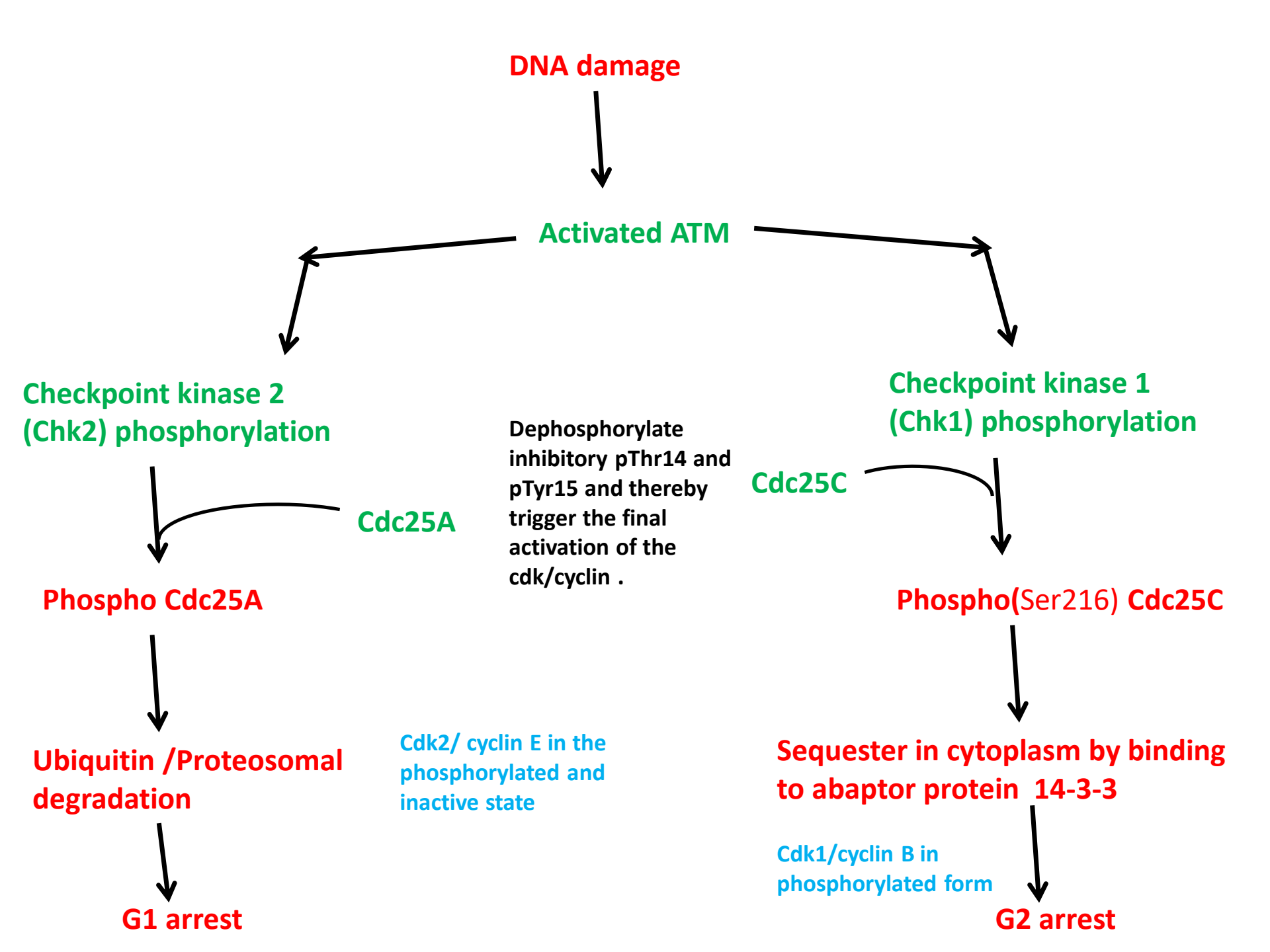
Figure 17-62 part 3 of 3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

High E2F, cyclin E is found in many cancers.
Defect in RB pathway(mutation in RB protein)

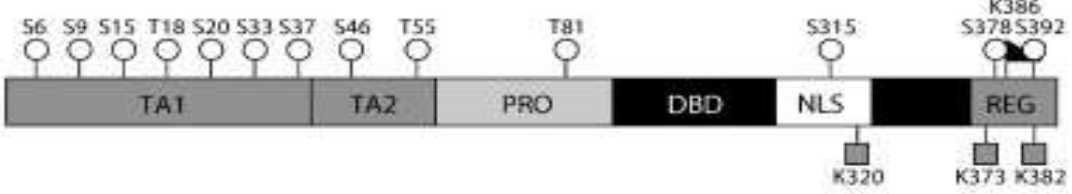
Phenotypic change after DNA damage – general strategy

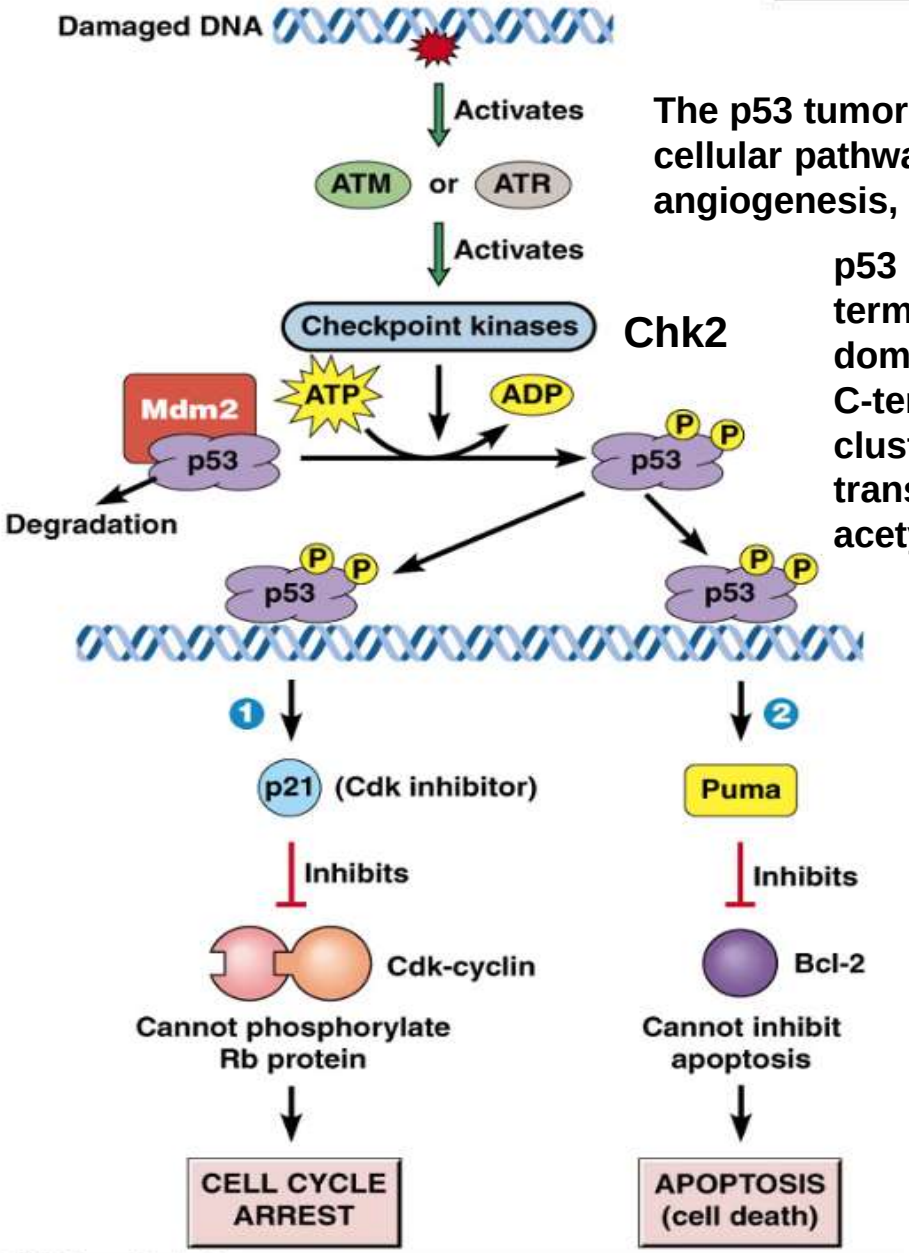





DNA damage
Activated ATM
Checkpoint kinase 2 (Chk2) phosphorylation
Checkpoint kinase 1 (Chk1) phosphorylation
Cdc25A
Cdc25C
Dephosphorylate inhibitory pThr14 and pTyr15 and thereby trigger the final activation of the cdk/cyclin .
Phospho Cdc25A
Phospho(Ser216) Cdc25C
Ubiquitin /Proteosomal degradation
Cdk2/ cyclin E in the phosphorylated and inactive state
Sequester in cytoplasm by binding to abaptor protein 14-3-3
Cdk1/cyclin B in phosphorylated form
G1 arrest
G2 arrest

P 53 pathway cell cycle check point.





G1 arrest

The p53 tumor suppressor is a key transcription factor regulating cellular pathways such as DNA repair, cell cycle, apoptosis, angiogenesis, and senescence

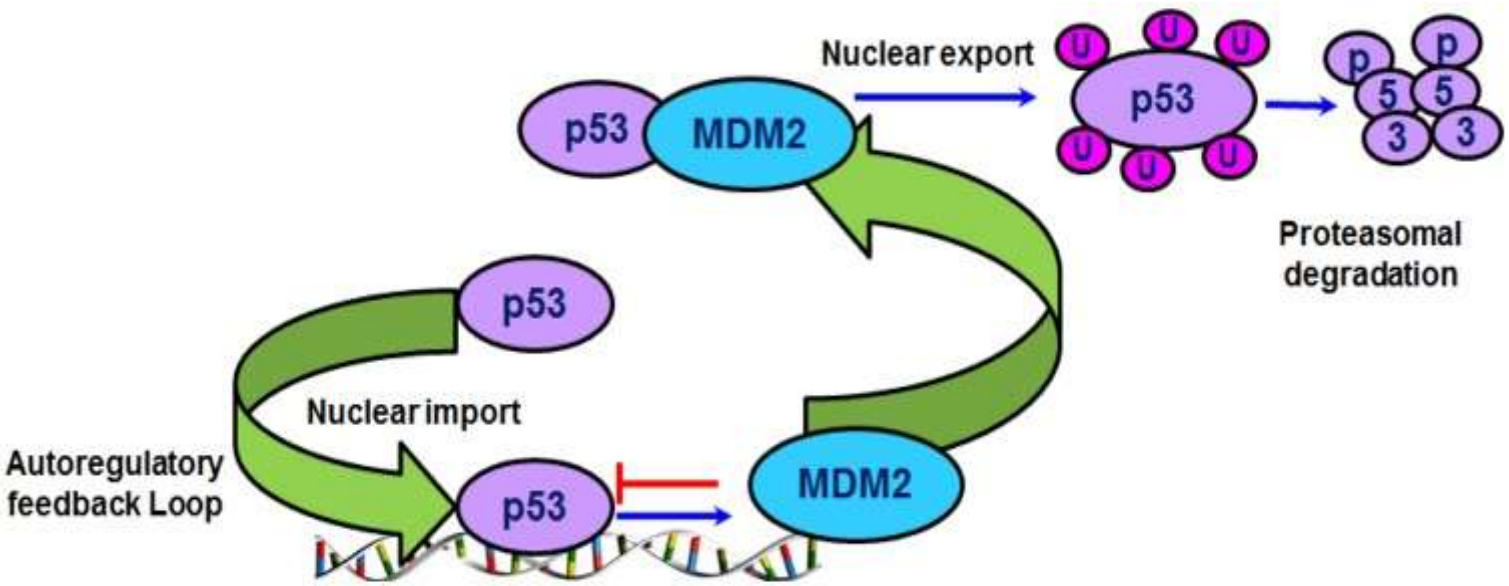
p53 is organized in several domains including two N-terminal transactivation domains (TA1, TA2), a proline-rich domain (PRO), a central DNA binding domain (DBD), and a C-terminal regulatory domain (REG). Multiple residues clustered at the N- and C-terminus of p53 are post-translationally modified by phosphorylation (circles), acetylation and ubiquitination (squares), and sumoylation

1. **Cell cycle progression is driven by phosphorylation events mediated by cyclin/cdk complexes. These cyclin/cdk complexes are thus the main targets of the effectors of the G1/S checkpoint .**

2. **Following activation by DNA damage, ATM Kinase activates CHK2 which inturn phosphorylate p53.Phospho p53 released from MDM2.**
3. **P53 accumulate on DNA and act transcription factor to transcribe p21, which in turn inhibit cycle E/CDK2 and prevent cell to enter S phase**

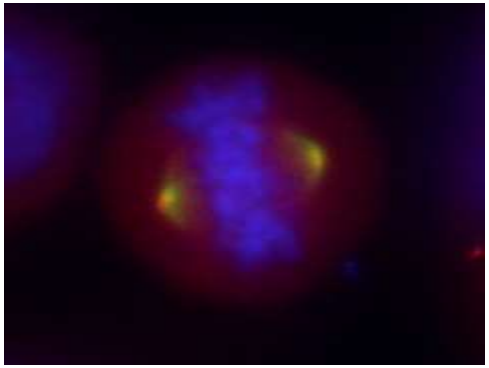
Expression of p21, even in the absence of p53, was sufficient to induce growth arrest at the G1

The traditional MDM2-p53 regulatory pathway.

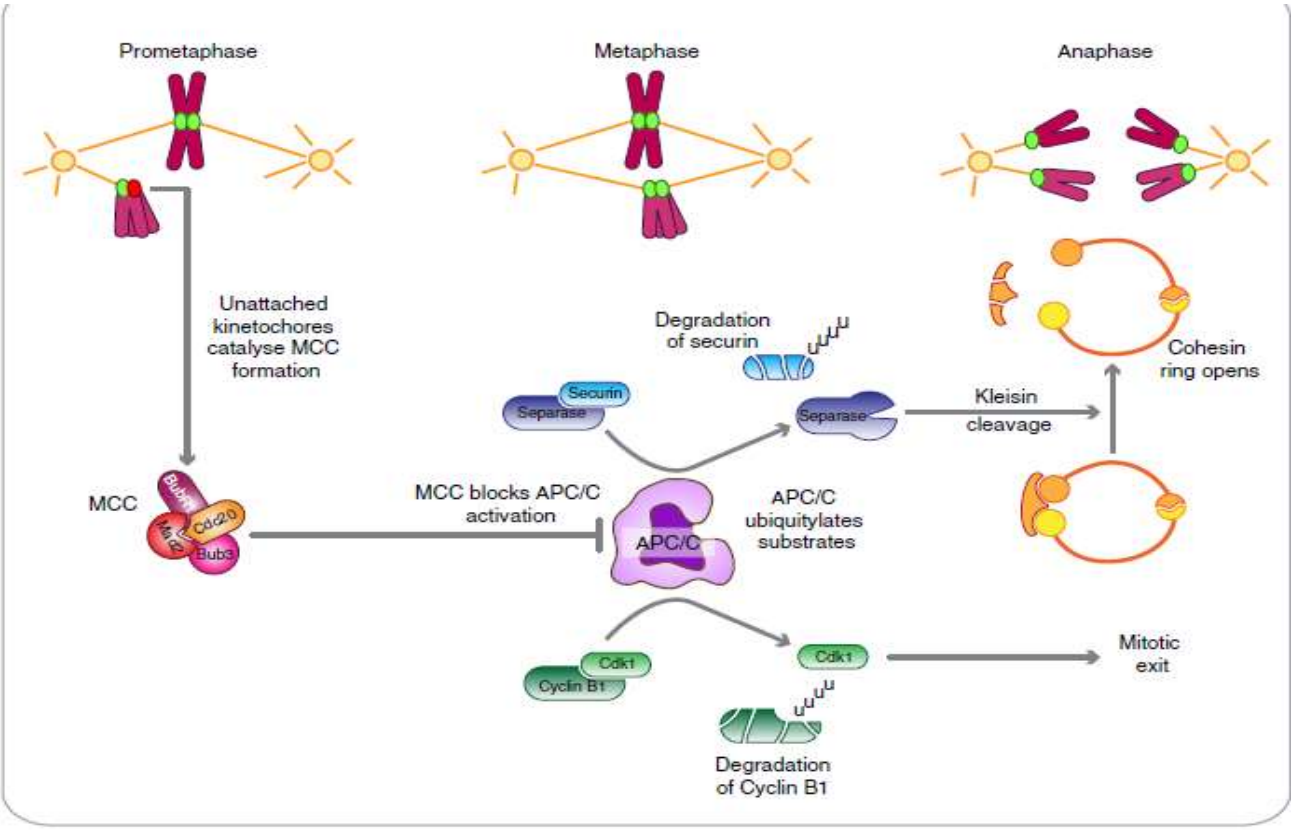


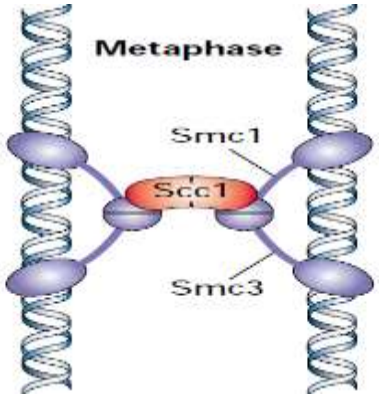
p53 regulates MDM2 oncoprotein expression by binding to its promoter. The increased MDM2 levels cause it, in turn, to bind and inactivate p53 by directly blocking the p53 transactivational domain and by targeting the p53 protein for ubiquitin-dependent degradation by the proteasome. This elegant autoregulatory loop helps to maintain low cellular levels of p53 in normal cells. The levels of p53 must be tightly controlled in unstressed cells since high levels of the anti-proliferative and pro-apoptotic p53 can be detrimental to normal cell growth and development.

Spindle Assembly Checkpoint- with in M phase



- How do unattached kinetochores generate the on signal **TO STOP** progression?
- How does the SAC inhibit anaphase onset?



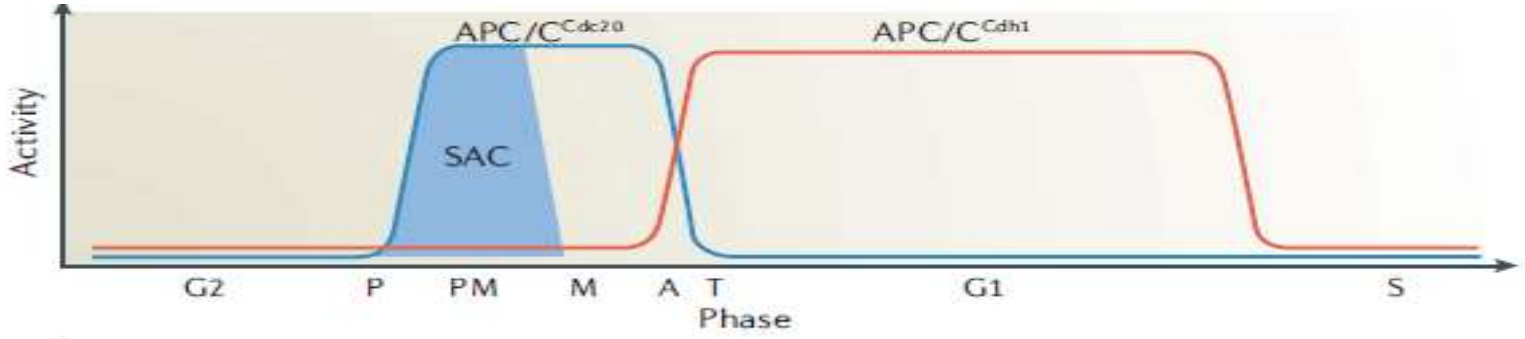


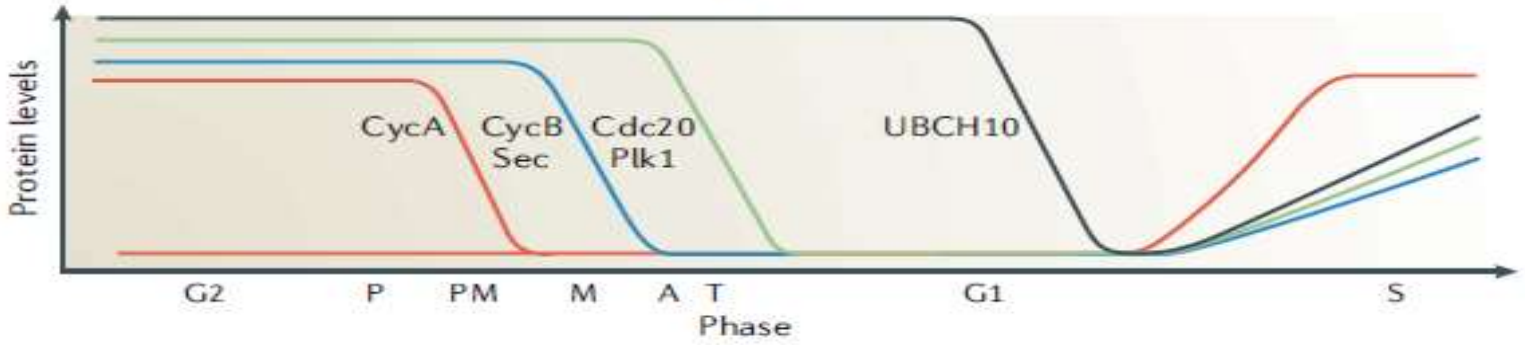
The multiprotein cohesin complex contains SMC1 and SMC3 (purple), dimeric proteins that bind DNA of each sister chromatid through globular domains at one end. Scc1 (orange) and two other cohesin subunits (not shown) bind to the SMC proteins associated with each chromatid, thus crosslinking the chromatids.

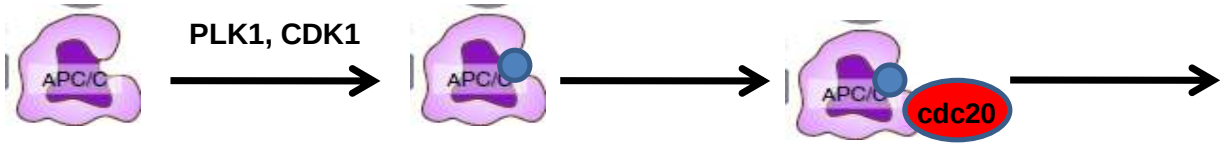
early stages of mitosis (prometaphase), unattached kinetochores catalyse the formation of the **mitotic checkpoint complex** (MCC) composed of BubR1, Bub3, Mad2 and Cdc20, leading to inhibition of the APC/C.

Once all the chromosomes are aligned with their kinetochores attached to the spindle (metaphase), generation of the MCC ceases, allowing Cdc20 to activate the APC/C, leading to the ubiquitylation and degradation of securin and cyclin B1.

Degradation of securin liberates separase which in turn cleaves the Scc1 kleisin subunit of the cohesin ring structure; this opens the ring, allowing sister chromatids to separate (anaphase). Meanwhile, degradation of cyclin B1 inactivates Cdk1,
leading to mitotic exit.



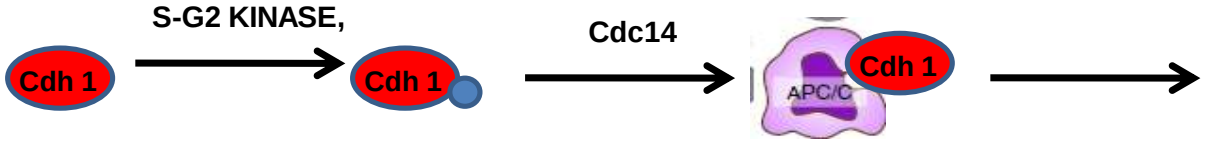




Promote proper chromosome segregation and prepare for mitotic exit, Cyclin A, securin degradation

Degradation of securin and all B type cyclin and mediates the destruction of additional substrates such as Polo-like kinase-1 (Plk1) and Cdc20, which leads to the inactivation of APC/Cdc20. enables a new round of DNA replication by permitting the assembly of pre-RCs in G1

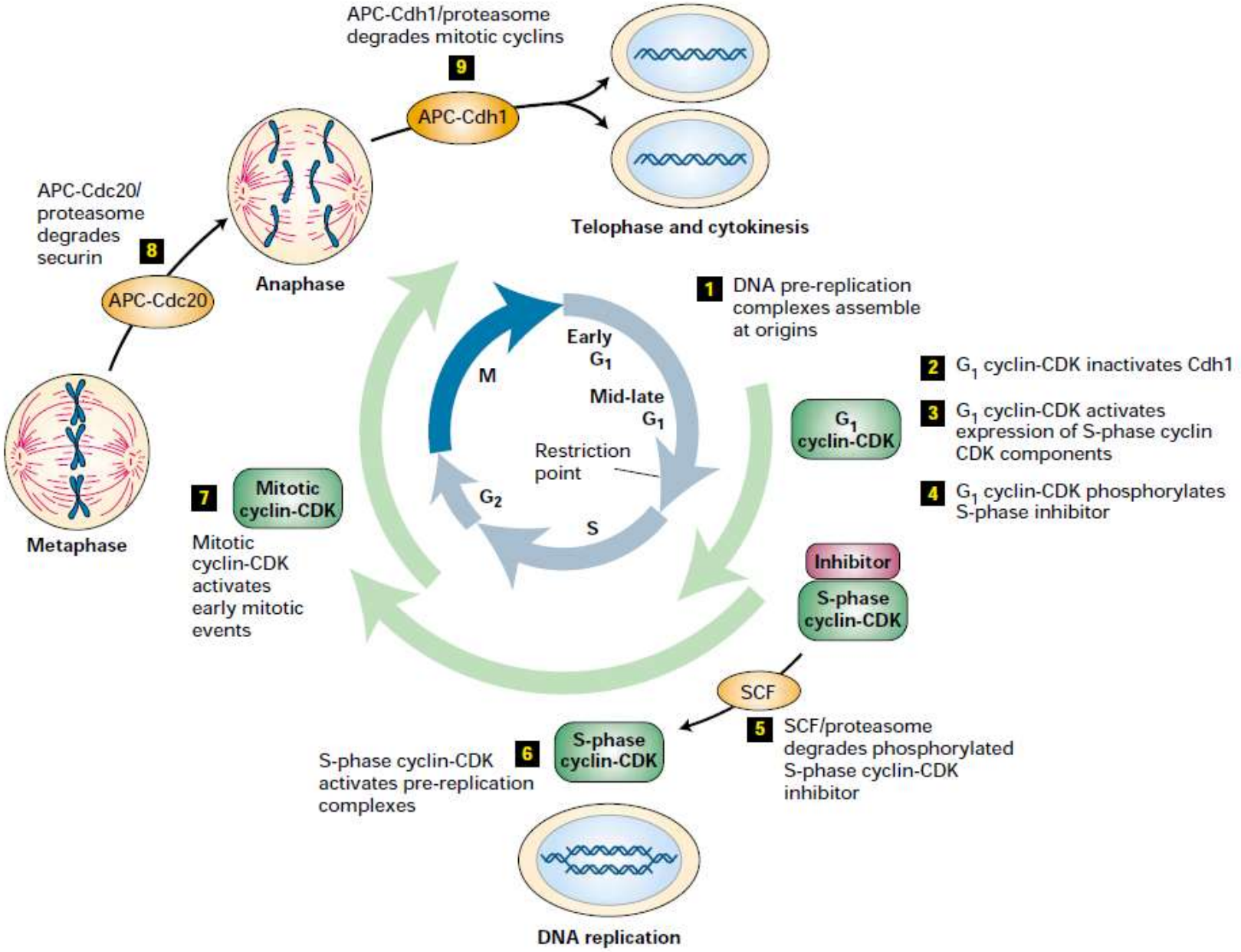
cyclin E–Cdk2 inactivates APC/Cdh1 by phosphorylating Cdh1.

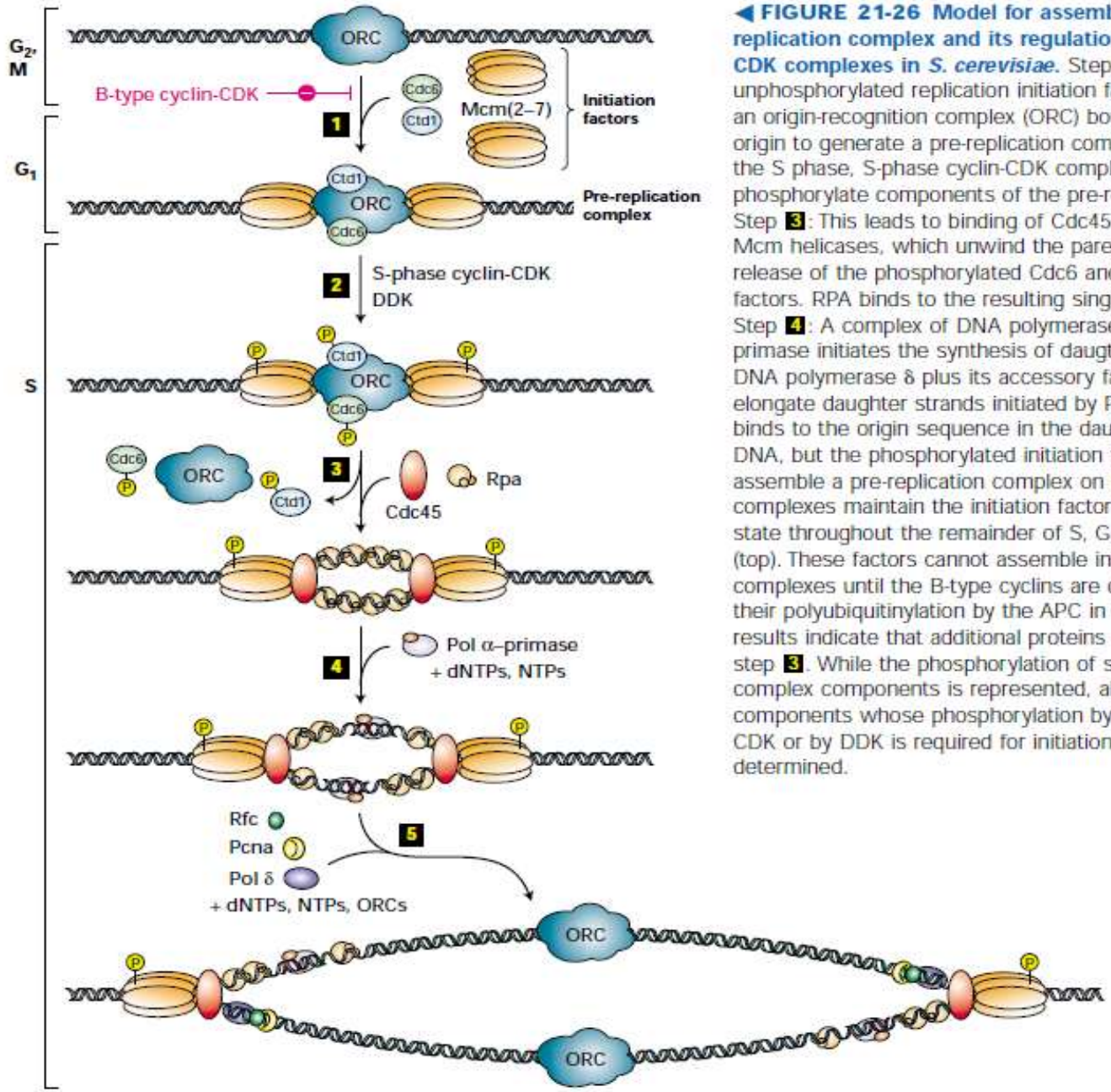


Activation of APC/C by Cdc20 and Cdh1 during the cell cycle.

Anaphase promoting complex/cyclosome Cdc20 (APC/CCdc20) is thought to be assembled in prophase (P) and initiates the degradation of cyclin A (CycA) already in prometaphase (PM). Proteolysis of cyclin B (CycB) and the separase inhibitor securin (Sec) also depends on APC/C/Cdc20 but is delayed until metaphase (M) by the spindle-assembly checkpoint (SAC).

During anaphase (A) and telophase (T), APC/Cdh1 is activated, contributes to the degradation of securin and cyclin B, and mediates the destruction of additional substrates such as Polo-like kinase-1 (Plk1) and Cdc20, which leads to the inactivation of APC/CCdc20. In G1 phase, APC/CCdh1 mediates the destruction of the ubiquitinconjugating (E2) enzyme UBCH10, and thereby allows for the accumulation of cyclin A, which contributes to the inactivation of APC/CCdh1 at the transition from G1 to S phase.


APC-Cdh1/proteasome degrades mitotic cyclins
9
APC-Cdh1
Telophase and cytokinesis
APC-Cdc20/ proteasome degrades securin
8
APC-Cdc20
Anaphase
Metaphase
1 DNA pre-replication complexes assemble at origins
Early G1
Mid-late G1
Restriction point
S
G2
M
2 G1 cyclin-CDK inactivates Cdh1
3 G1 cyclin-CDK activates expression of S-phase cyclin CDK components
4 G1 cyclin-CDK phosphorylates S-phase inhibitor
G1 cyclin-CDK
Inhibitor
S-phase cyclin-CDK
SCF
5 SCF/proteasome degrades phosphorylated S-phase cyclin-CDK inhibitor
S-phase cyclin-CDK
6 S-phase cyclin-CDK activates pre-replication complexes
DNA replication
7
Mitotic cyclin-CDK
Mitotic cyclin-CDK activates early mitotic events



◀ FIGURE 21-26 Model for assembly of the pre-replication complex and its regulation by S-phase cyclin-CDK complexes in *S. cerevisiae*. Step **1**: During early G_1, unphosphorylated replication initiation factors assemble on an origin-recognition complex (ORC) bound to a replication origin to generate a pre-replication complex. Step **2**: In the S phase, S-phase cyclin-CDK complexes and DDK phosphorylate components of the pre-replication complex. Step **3**: This leads to binding of Cdc45, activation of the Mcm helicases, which unwind the parental DNA strands, and release of the phosphorylated Cdc6 and Ctd1 initiation factors. RPA binds to the resulting single-stranded DNA. Step **4**: A complex of DNA polymerase α (Pol α) and primase initiates the synthesis of daugther strands. Step **5**: DNA polymerase δ plus its accessory factors PCNA and Rfc elongate daughter strands initiated by Pol α-primase. ORC binds to the origin sequence in the daughter double-stranded DNA, but the phosphorylated initiation factors cannot assemble a pre-replication complex on it. B-type cyclin-CDK complexes maintain the initiation factors in a phosporylated state throughout the remainder of S, G_2, and early anaphase (top). These factors cannot assemble into new pre-replication complexes until the B-type cyclins are degraded following their polyubiquitinylation by the APC in late anaphase. Recent results indicate that additional proteins not shown function at step **3**. While the phosphorylation of several pre-replication complex components is represented, all the critical components whose phosphorylation by an S phase cyclin-CDK or by DDK is required for initiation have not been determined.