

# MOLECULAR CELL BIOLOGY

SIXTH EDITION

## CHAPTER 20

### Regulating the Eukaryotic Cell Cycle

Cell Cycle

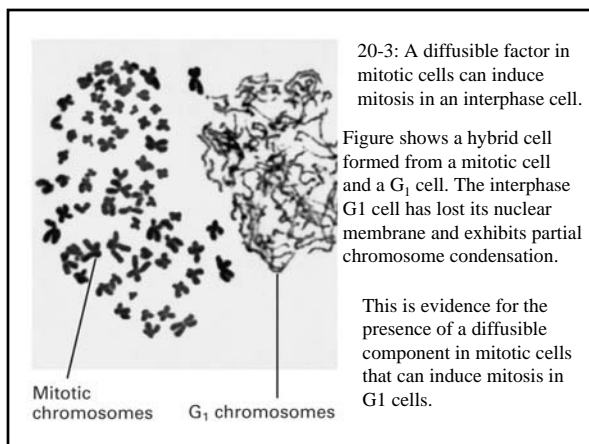
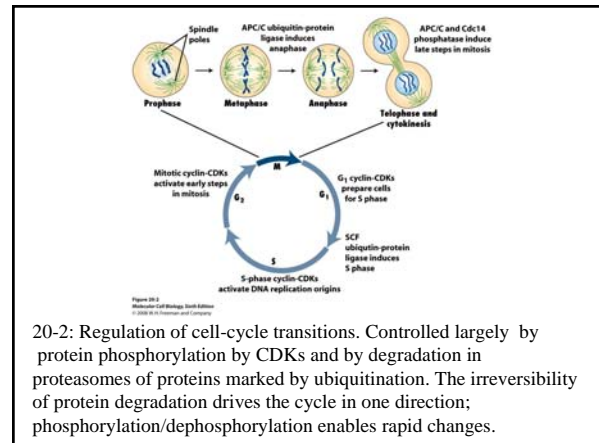
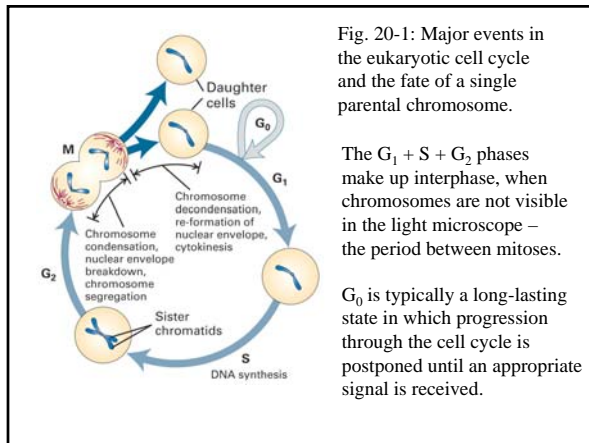
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#### Section 20.1:

#### Overview of the Eukaryotic Cell Cycle and Its Control (note how organisms as diverse as yeast, sea urchins and frogs (*Xenopus*) have contributed in diverse ways)

1. Four phases: M (mitosis),  $G_1$  (first gap phase), S (DNA synthesis),  $G_2$  (second gap phase).
2. Regulatory cyclin proteins and catalytic cyclin-dependent kinases regulate cell cycle progression. Protein phosphorylation (and dephosphorylation) and protein degradation are critical to cell cycle regulation.
3. Chromosome replication and segregation are fundamentally similar in all eukaryotes, yeast to human. Controlled by heterodimeric protein kinases (cyclin/CDKs).



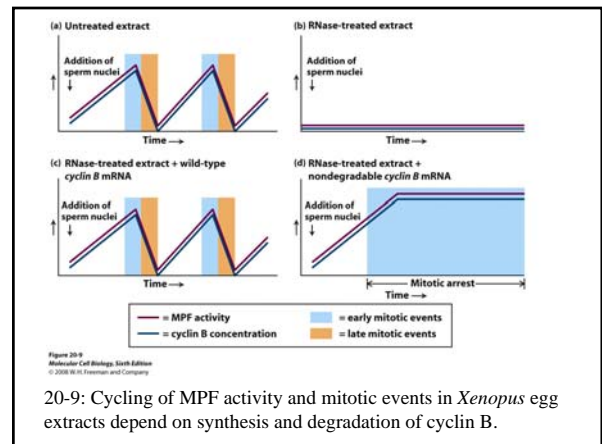
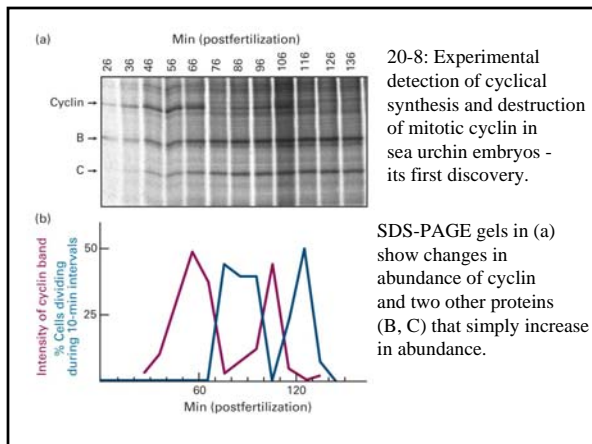
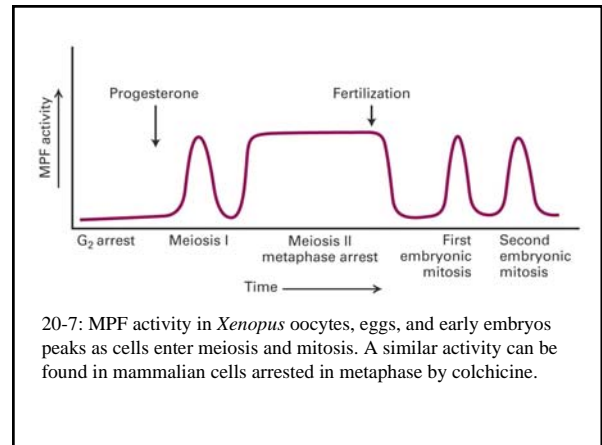
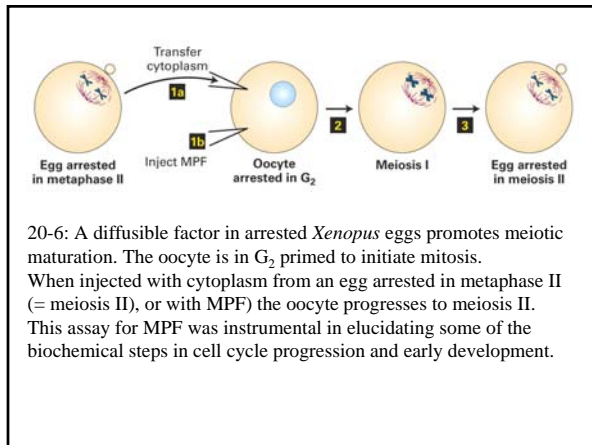
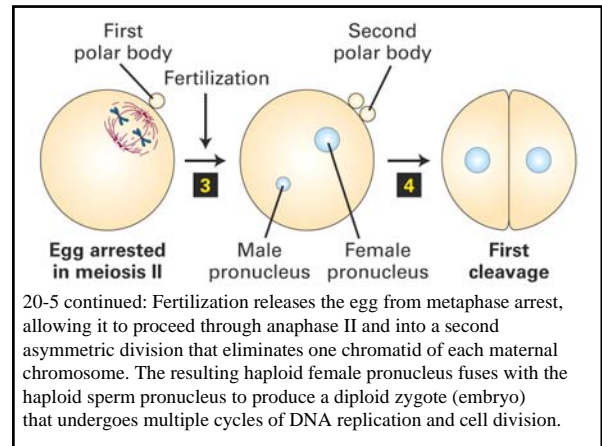
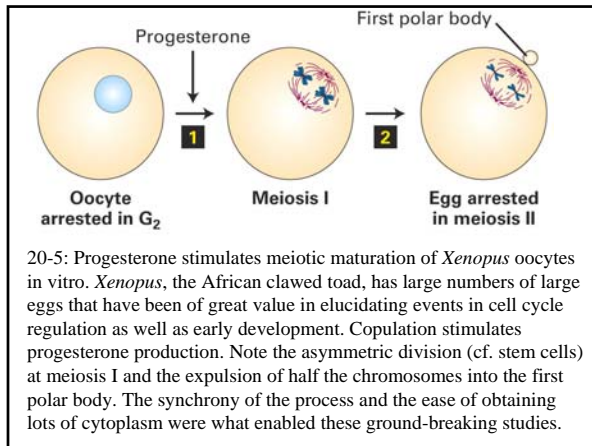
#### Section 20.2: Control of Mitosis by Cyclins and MPF Activity

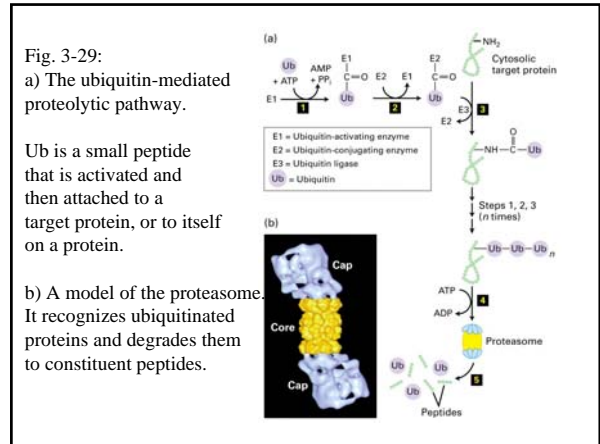
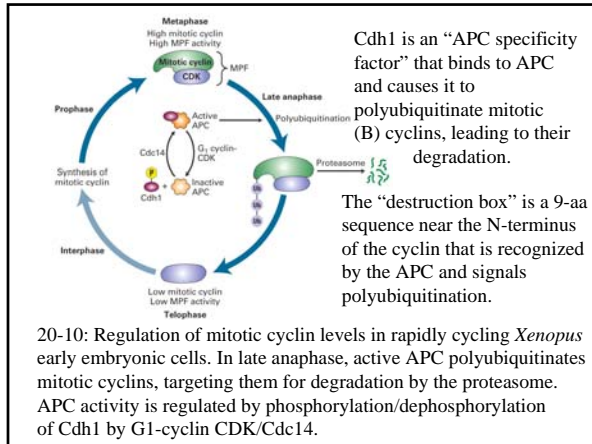
Maturation promoting factor (MPF) is a protein kinase (requiring a mitotic cyclin) that stimulates mitosis, and hence is also called mitosis promoting factor.

The increase and decrease in MPF activity during the cell cycle in the early embryo reflects the cyclic synthesis and degradation of mitotic cyclins.

The anaphase-promoting complex/cyclosome (APC/C) is a **ubiquitin ligase** that recognizes the **destruction box** sequence in mitotic cyclins, marking them for degradation in late anaphase and thus terminating mitosis.

Deactivation of APC/C (by  $G_1$ -cyclin/CDK) in  $G_1$  permits mitotic (and S phase) cyclins to accumulate, allowing the cell cycle to continue.



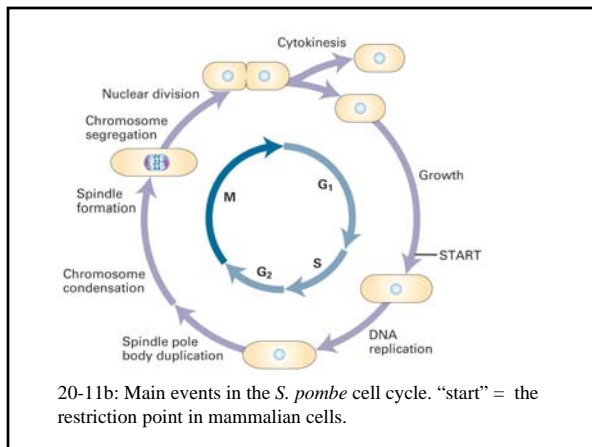
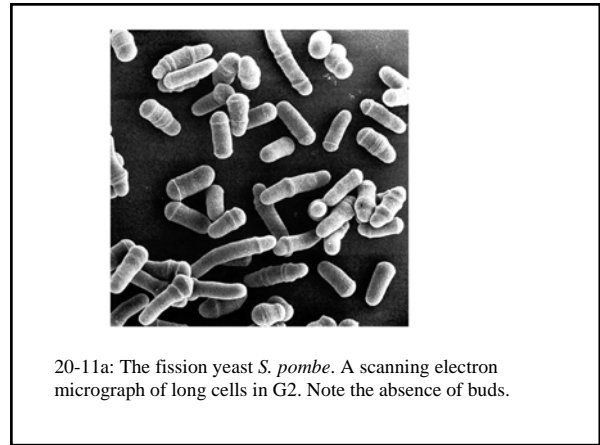


**Section 20.3:**  
**Cyclin-dependent kinase regulation during mitosis**

In the fission yeast *S. pombe* cdc2 protein kinase is activated by associating with the cdc13 cyclin (= MPF activity). There is only one CDK in yeast; it associates with different cyclins.

Activity of the mitotic cyclin-CDK depends on the phosphorylation state of two amino acids in the CDK subunit: Phosphorylation of thr161 activates, phosphorylation of tyr15 inhibits. This inhibitory phosphate is removed by the Cdc25 protein phosphatase.

A decrease in Wee1 kinase activity and an increase in cdc25 phosphatase activity initiates mitosis.

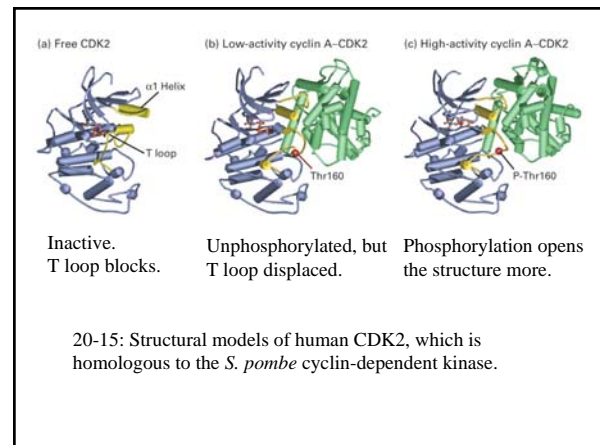
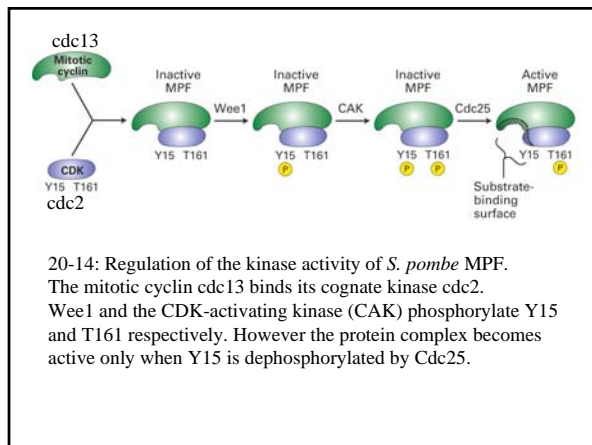
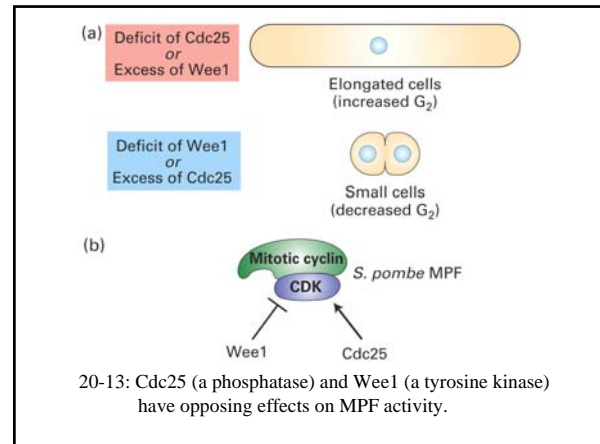
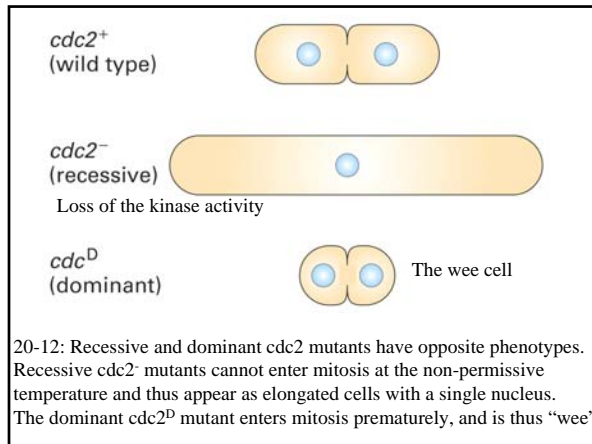


**Temperature-sensitive mutations**

These are mutations that give rise to mutant proteins that exhibit an increased (or decreased) sensitivity to temperature.

For example, a change from one amino acid to another at a critical location in the protein can result in a protein that is unstable at 37°C, the “nonpermissive temperature”. Thus if that protein is essential for normal cell growth, then the cell will not grow. Reducing the temperature to 30°C, the “permissive temperature”, may allow the cell to grow.

Cdc mutants fail to progress through the cell cycle; wee mutants are defective in proteins that prevent cells from dividing prematurely (check points).

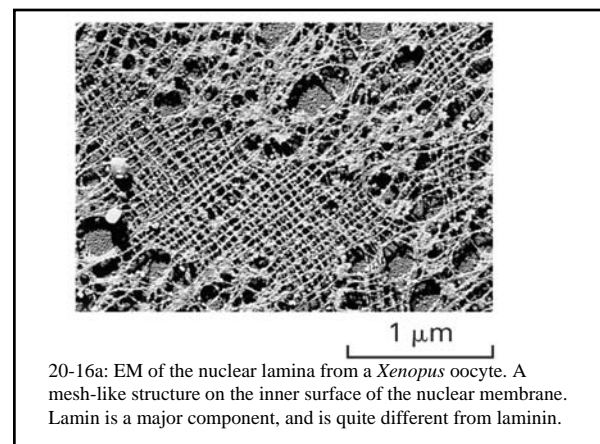


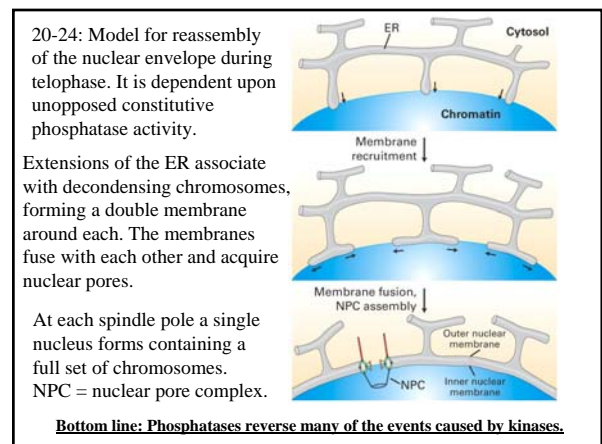
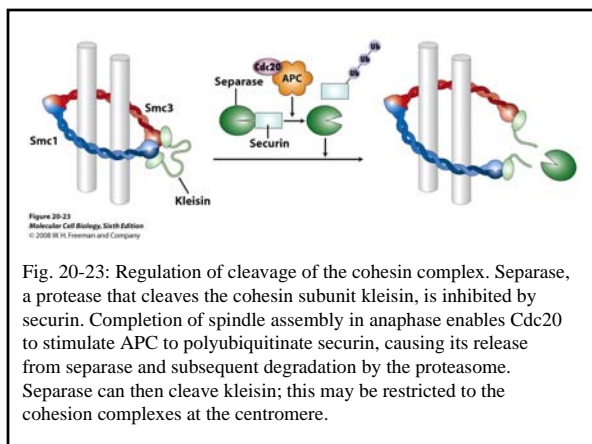
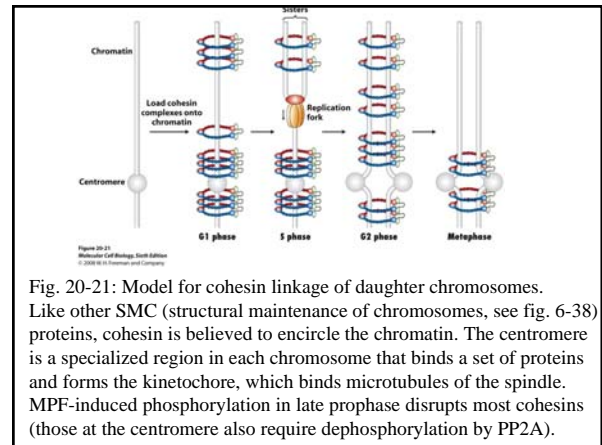
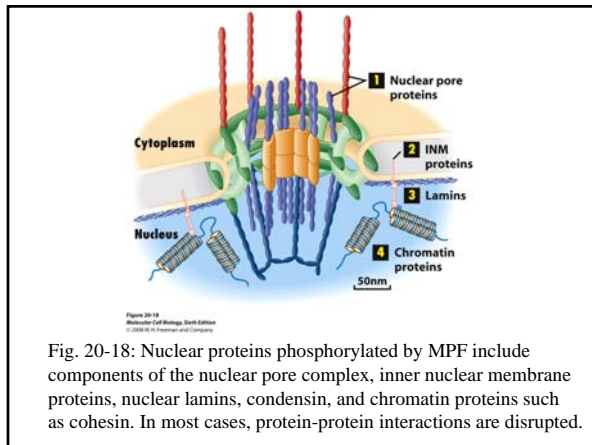
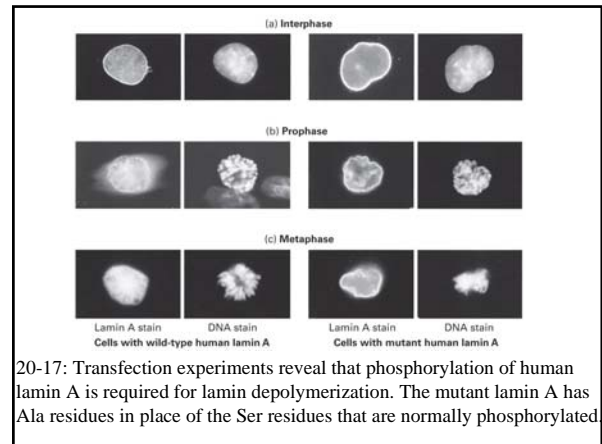
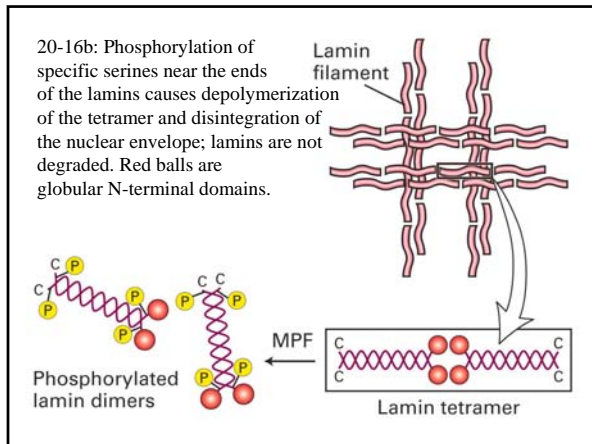
**Section 20.4**  
**Molecular Mechanisms Regulating Mitotic Events**

Early in mitosis MPF-catalyzed phosphorylation of the lamins and other proteins such as **Condensin** results in disassembly of the nuclear envelope and chromosome condensation.

At anaphase, Cdc20/APC polyubiquitinates **Securin**, resulting in its degradation; this activates **Separase** and cleaves a **Cohesin** subunit (kleisin), unlinking sister chromatids.

After chromatid separation, mitotic cyclins are ubiquitinated (by APC/Cdh1) and degraded. The decline in MPF activity allows constitutive protein phosphatases like Cdc14 to remove regulatory phosphates, allowing chromosome decondensation and reformation of the nucleus.

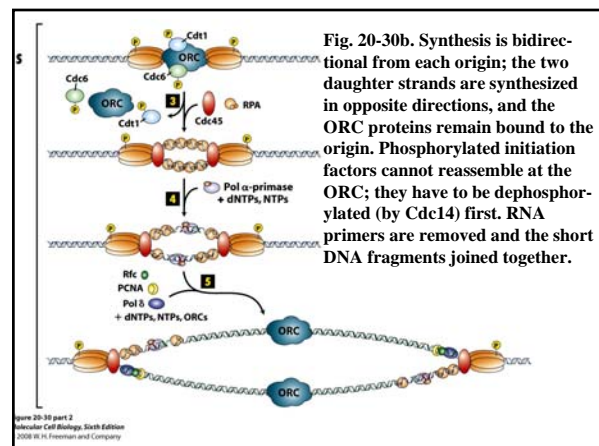
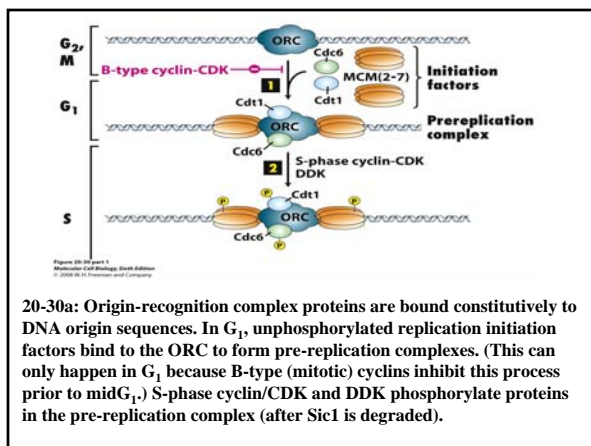
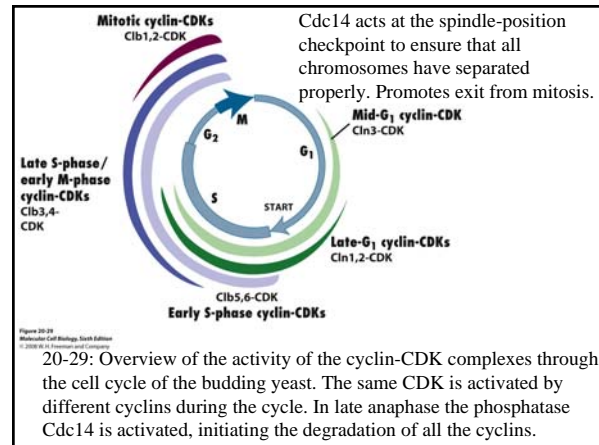
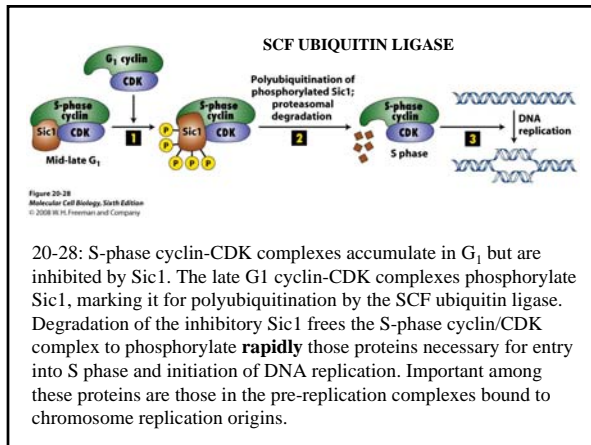
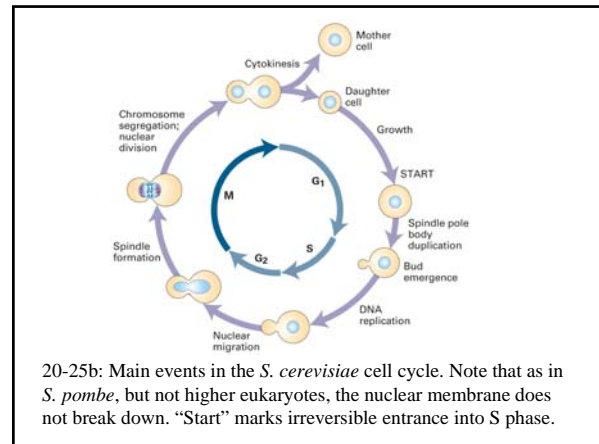




**Section 20.5**  
**Cyclin-CDK and Ubiquitin-Protein Ligase Control of S phase**

The budding yeast *S. cerevisiae* expresses a single CDK (CDC28) that interacts with three different cyclins during the cell cycle. G1 cyclins associate with CDK to form S phase-promoting factors that phosphorylate and regulate proteins required for DNA synthesis. "Start" is a point in the cycle at which further progression of the cell cycle becomes independent of nutrients.

DNA replication is initiated from pre-replication complexes assembled at origins during early G1. S-phase cyclin-CDK complexes trigger initiation from pre-replication complexes and inhibit assembly of new pre-replication complexes. Initiation of DNA replication at each origin occurs once, and only once, during the cell cycle.



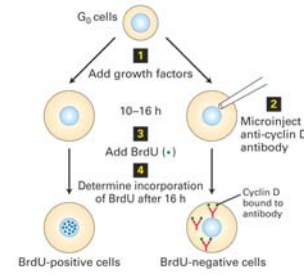
**Section 20.6: Cell-cycle Control in Mammalian Cells**

Growth factors (mitogens) usually are required to drive mammalian cells into proliferation by stimulating synthesis of transcription factors, cyclins, and cdk. Once past the restriction point, these factors are not required.

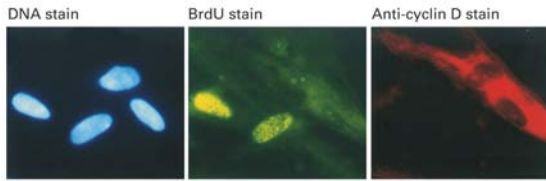
Unphosphorylated Rb (retinoblastoma protein) binds E2Fs forming transcriptional repressors. Phosphorylation of Rb by cyclin D–CDK4/6 liberates E2Fs to stimulate expression of genes necessary for S phase progression. Cyclin E/CDK2 reinforces this activation.

Cyclin A-CDK2 activates pre-replication complexes to initiate DNA synthesis. Its activity is controlled by phosphorylation, CDK-inhibitory proteins (CIPs), and proteasome action.

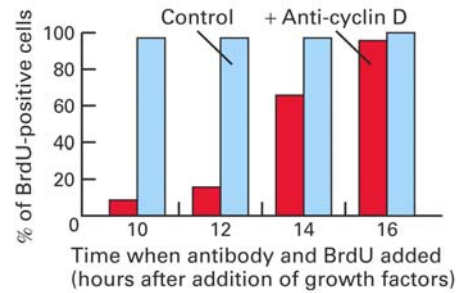
Cyclin A/B-CDK1 drives cells into mitosis and are then polyubiquitinated by the anaphase promoting complex (APC) and degraded by the proteasome.



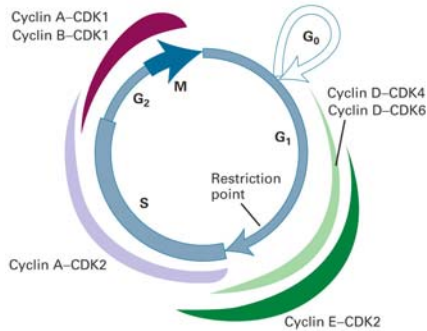
20-31a: Evidence that cyclin D is required for passage through the restriction point. BrdU is bromodeoxyuridine, a thymidine analog.



20-31b: Same four cells are in each field of view. DNA stain shows the nucleus. BrdU “stains” only nuclei that have initiated DNA synthesis. Anti-cyclin D stain shows the cells injected with antibody; in these cells BrdU incorporation was suppressed.



20-31c: Anti-cyclin D antibody must be added before 14 h.



20-32: Activity of mammalian cyclin-CDK complexes through the course of the cell cycle in cultured G<sub>0</sub> cells induced to divide by treatment with growth factors.

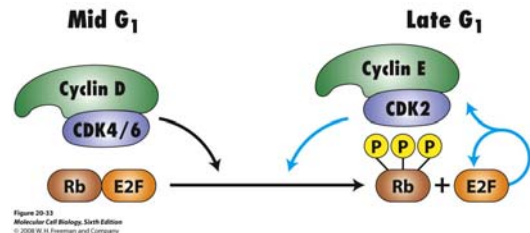


Figure 20-33 Molecular Cell Biology, Sixth Edition © 2008 W. H. Freeman and Company

20-33: Regulation of Rb and E2F activities in mid-late G<sub>1</sub>. Cyclin D and CDK4/6 initiate phosphorylation in midG<sub>1</sub>; this leads to activation of cyclin E and CDK2, which further activates Rb via additional phosphorylation events, thereby enhancing E2F expression. E2F activates (by phosphorylation) itself, Rb, and other transcription factors that stimulate transcription of genes required for DNA replication.

**CDK inhibitory proteins (CIPs) and inhibitors of kinase 4 (INK4s) represent another level of control of the cell cycle**

p21<sup>CIP</sup>, p27<sup>KIP1</sup>, p57<sup>KIP2</sup>

– inhibit the activity of cyclinA-CDK2, blocking progression into S phase. Other CDKs are also inhibited with varying specificity.

INK4, also called p16, interacts only with CDK4 and CDK6, blocking their interaction with cyclin D and hence progression through G<sub>1</sub>. It is one of the important tumor suppressors.

**Section 20.7: Checkpoints in Cell Cycle Regulation**

Checkpoint controls function to ensure that critical stages of the cell cycle are completed before the following stage is initiated. (See Table 20-2)

Intra-S-phase checkpoint: Cdc25c blocked from activating CDK1 by Chk1/ATR, which is activated by replication forks.

Spindle-assembly checkpoint: blocks anaphase initiation via Mad2 inhibition of APC/Cdc20 required for securin degradation.

Spindle position checkpoint: blocks telophase/cell division by inhibiting the Cdc14 (CdcA?) phosphatase-induced activation of B-type cyclins. (Cdc14 remains sequestered in nucleoli.)

DNA damage checkpoints block initiation or progression of DNA replication in response to DNA damage. Involved are ATM/R kinases, p53 transcription factor, p21<sup>CIP</sup> cyclin inhibitor and Chk1/2 inhibition of Cdc25A.

