

G2 Checkpoint

- Check for:
- Cell size
 - DNA damage

Spindle Assembly Checkpoint

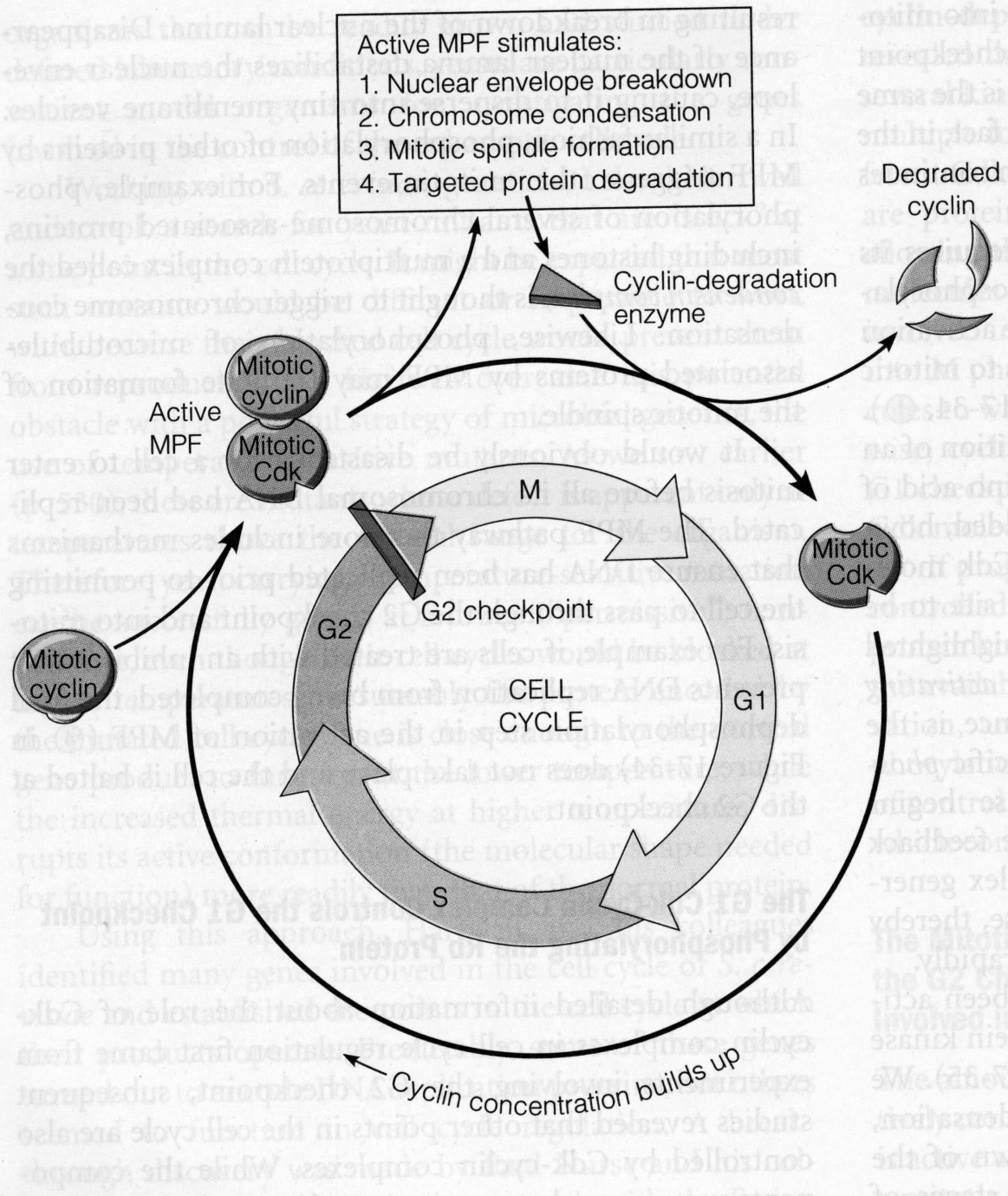
- Check for:
- Chromosome attachment to spindle

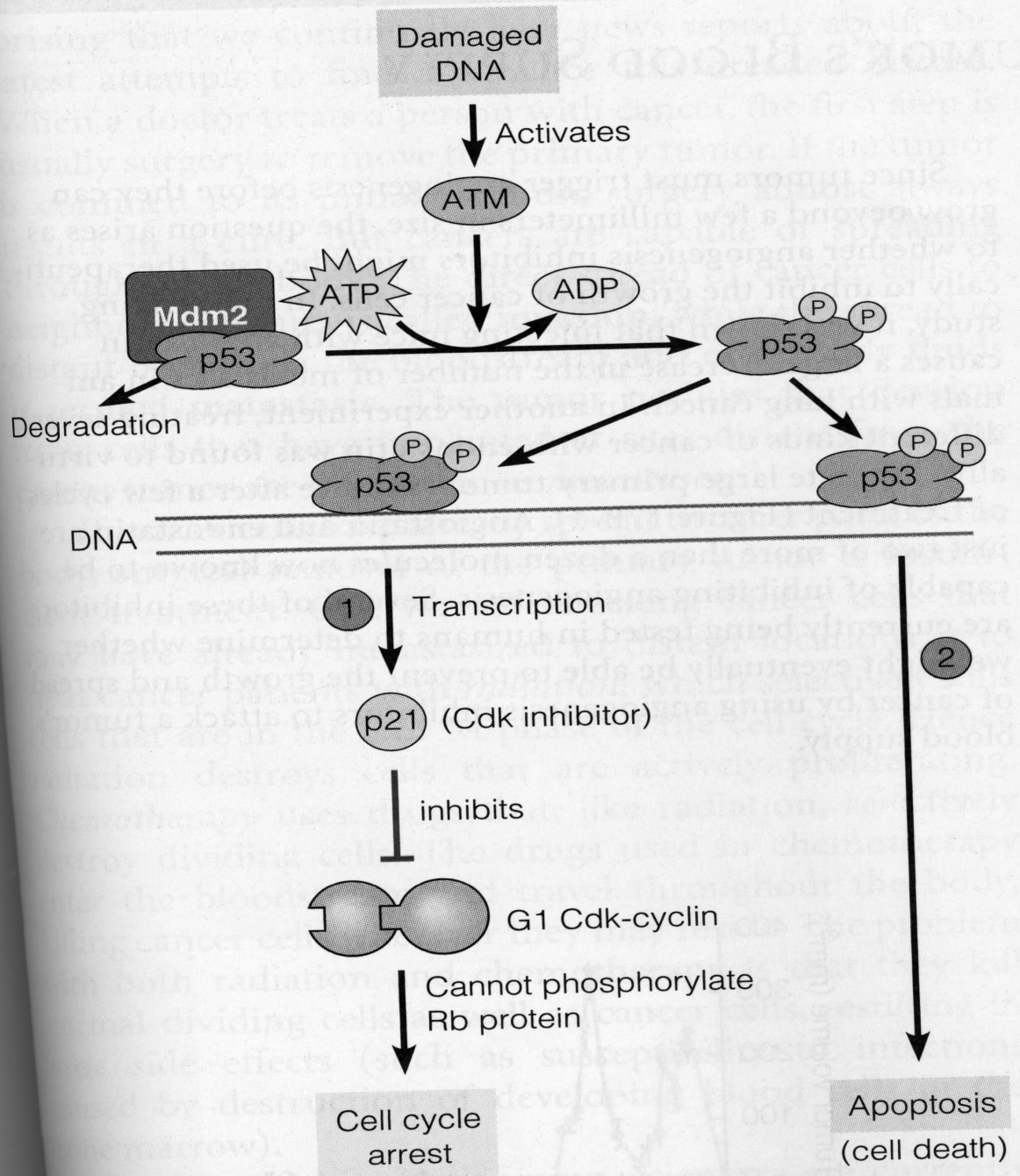
G1 Checkpoint

- Check for:
- Cell size
 - Nutrients
 - Growth factors
 - DNA damage

Resting state

Resting state (G0)





- Control system must accomplish several tasks:
 - Ensure that the events associated with each phase of the cell cycle are carried out at the appropriate time and in the appropriate sequence
 - Make sure that each phase of the cycle has been properly completed before the next phase is initiated.
 - Control system must be able to respond to external conditions that indicate the need for cell growth and division)

- Conditions within the cell determine whether or not the cell will proceed to the next stage of cycle
 - First check point late during G1 = restriction point: progression through restriction point determined by growth factors present. If successful the cell will go into S, if not cell stays in G1 or G0
 - G2 checkpoint located at boundary between G2 and M, proper completion of DNA synthesis is required before cell begins mitosis
 - 3rd point is the spindle assembly check point and is at the junction between metaphase and anaphase: all chromosomes must be attached to spindle and properly aligned

- Experiments done using HETEROKARYONS:
two cells in different stages of cell cycle are fused to form a single cell with two nuclei
 - If one cell is in S and the other in G1 the G1 nucleus in the heterokaryon initiates DNA synthesis even if it would not normally have reached S phase until many hours later (molecules are present that trigger these events)

- **Mitosis-Promoting Factor (MPF)**
 - If cytoplasm taken from a mature egg cell is injected into the cytoplasm of an immature oocyte, the oocyte immediately begins meiosis– cytoplasmic signal named “maturation-promoting factor”
 - Subsequent experiments showed that MPF can also trigger mitosis when injected into fertilized from eggs
 - MPF is able to trigger passage through the G2 checkpoint in both mitosis and meiosis and the term now stands for mitosis-promoting factor (or M-phase promoting factor)

- Gene identified= cdc2-- activity of this gene is essential for the initiation of mitosis (i.e., passing through the G2 checkpoint)
 - **Cdc stands for cell division cycle**
 - The protein encoded by the cdc2 gene is one of two proteins making up MPF
- Protein encoded by the cdc2 gene functions as a protein kinase
 - Protein is active only when bound to a member of another group of proteins= cyclins. Cdc2 gene product is a cyclin-dependent kinase (Cdk).
 - **Cyclin levels fluctuate in cells and this acts as one level of regulation for activity of the cdc2 gene product**
 - **2nd regulation level involves amount of**

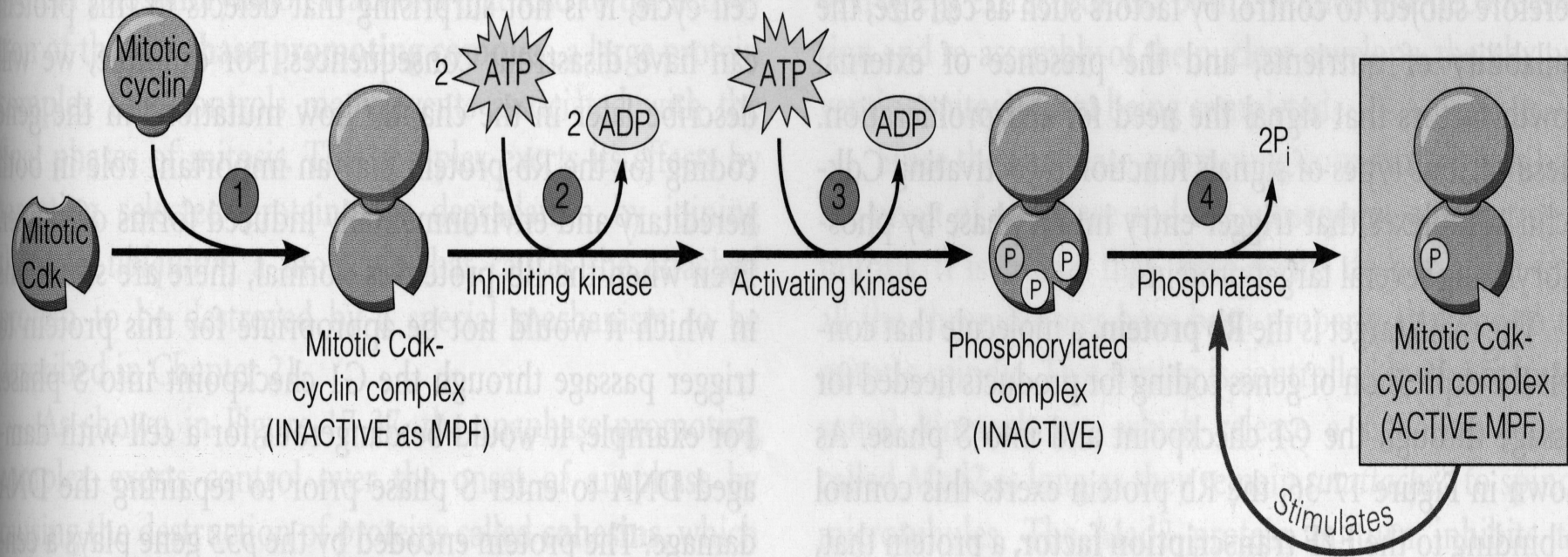


Figure 17-34 Phosphorylation and

Dephosphorylation in the Activation of a Cdk-Cyclin Complex. The series of reactions shown here for the formation of active MPF (mitotic Cdk-cyclin complex) was worked out using a combination of data from yeasts and frog eggs. ① The mitotic Cdk and

cyclin proteins form an inactive complex.

② An inhibiting kinase adds two phosphate groups (white) to the complex, which block its active site. ③ An activating kinase phosphorylates a third site on the complex (yellow phosphate). ④ A phosphatase removes the inhibiting phosphate groups,

converting the complex to a singly phosphorylated form, which is active as MPF. This active MPF in turn stimulates the phosphatase to produce additional active MPF. The result is a burst of MPF activity.

- Mitotic Cdk is active as a protein kinase only after it becomes bound to a mitotic cyclin
 - Mitotic Cdk remains constant throughout cycle
 - Concentration of mitotic cyclin gradually increases during the G1, S, and G2 phases of cycle, eventually reaching a critical threshold that allows activation of mitotic Cdk at end of G2
 - Cdk + cyclin = MPF complex (active) → triggers passage through G2 check point into mitosis

- To trigger mitosis, the complex requires the addition of an activating phosphate group to a specific amino acid of the Cdk molecule
 - Before this phosphate is added: an inhibiting kinase phosphorylates the Cdk molecule at 2 locations, causing active site to be blocked. The activating phosphate group is added and the two blocking phosphates are removed by a phosphatase.
 - The activated Cdk-cyclin complex generated stimulates the phosphatase, causing the activation process to proceed more rapidly

- Other Check points

- Much work done on the G2 checkpoint but other check points also involved in regulating cell cycling

- These other checkpoints controlled by Cdk-cyclin complexes as well

- There are a number of different Cdk and cyclin molecules that interact with each other- in different combinations and at different times in cell cycle

- Cdk-cyclin complexes regulated by:

- ❖ Different kinds of cyclins are synthesized and degraded during different phases of the cell cycle

- ❖ Activity of Cdk-cyclin complexes is controlled by phosphorylation and dephosphorylation reactions catalyzed by protein kinases and phosphatases

- G1 Checkpoint

- Passing from G1 → S is the main step that commits a cell to the process of cell division

- Controlled by cell size, availability of nutrients, and presence of growth factors

- Signals (above) control response through activation of Cdk-cyclin complexes that trigger entry into S by phosphorylating several target proteins

- Main target protein is Rb

- Rb controls the expression of genes that code for products needed to pass from G1 checkpoint into S

- Rb binds to the E2F transcription factor - when Rb is absent the E2F factor activates the transcription of genes coding for enzymes and other proteins required for DNA replication

- Rb inactivates the E2F factor
 - Rb activity controlled by Cdk-cyclin complexes that phosphorylate the Rb protein- when Rb phosphorylated it cannot bind to E2F
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- How might mutations in the Rb protein lead to cancer?

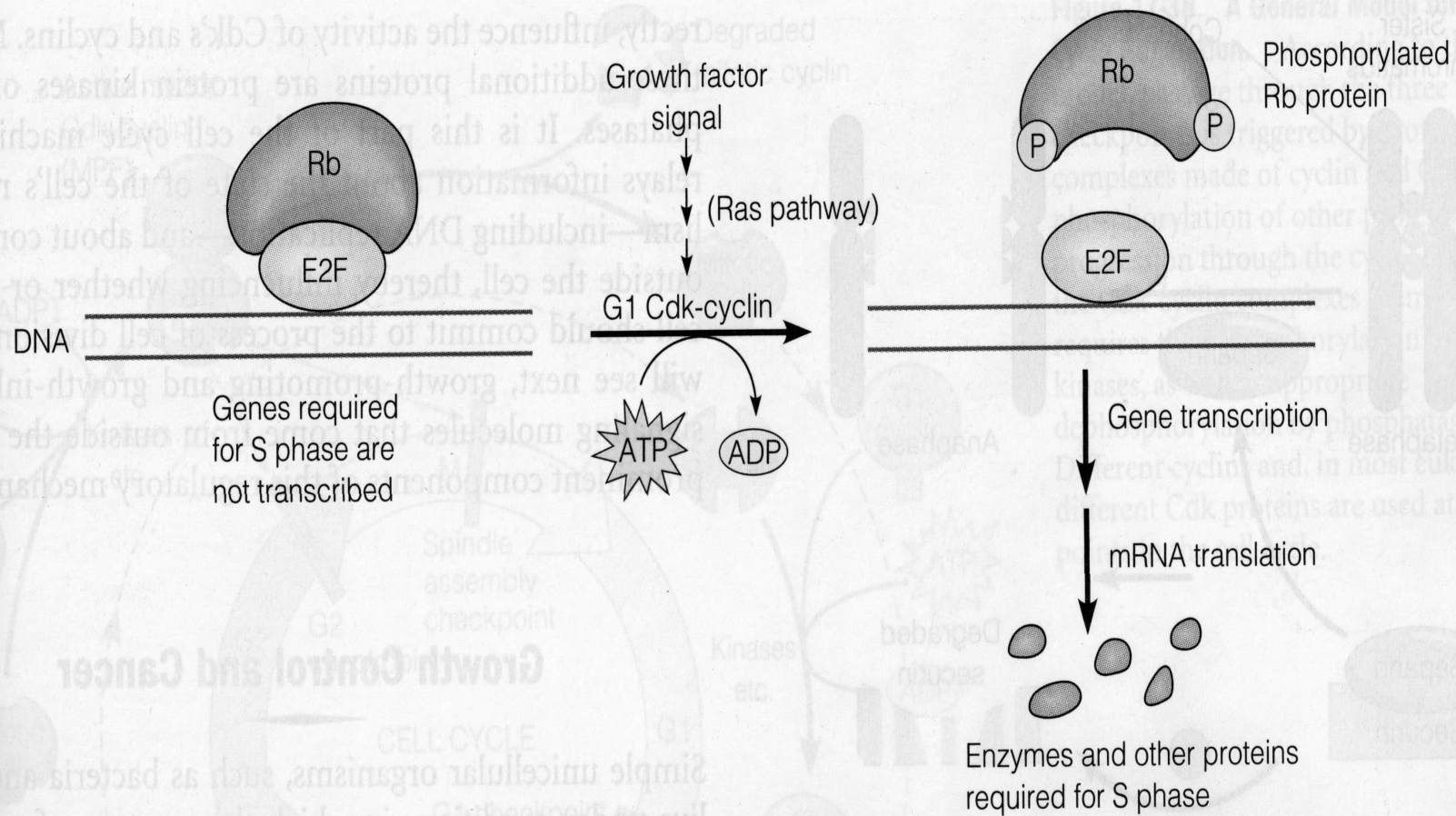


Figure 17-36 Role of the Rb Protein in Cell Cycle Control.

In its normal, dephosphorylated state, the Rb protein binds to the E2F transcription factor. This binding prevents E2F from activating the transcription of genes coding for proteins required for DNA replication, which are

needed before the cell can pass through the G1 checkpoint into S phase. In cells that have been stimulated by growth factors, the Ras pathway is activated (see Figure 17-39), which leads to the production and activation of a G1 Cdk-cyclin complex that catalyzes the phosphorylation of the Rb

protein. Phosphorylated Rb can no longer bind to E2F, thereby allowing E2F to activate gene transcription and trigger the onset of S phase. During the subsequent M phase (not shown), the Rb protein is dephosphorylated so that it can once again inhibit E2F.

- **p53** protein stops cells with damaged DNA from going through the G1 checkpoint by inhibiting the normal pathway for phosphorylating Rb
 - p53 increased in cells with DNA damage
 - Damaged DNA triggers activation of a protein kinase = **ATM**. ATM leads to phosphorylation of p53.
 - Phosphorylation of p53 prevents its interaction with **Mdm2**, a protein that promotes degradation of p53 by linking it with **ubiquitin** (targets proteins for degradation by proteasomes).

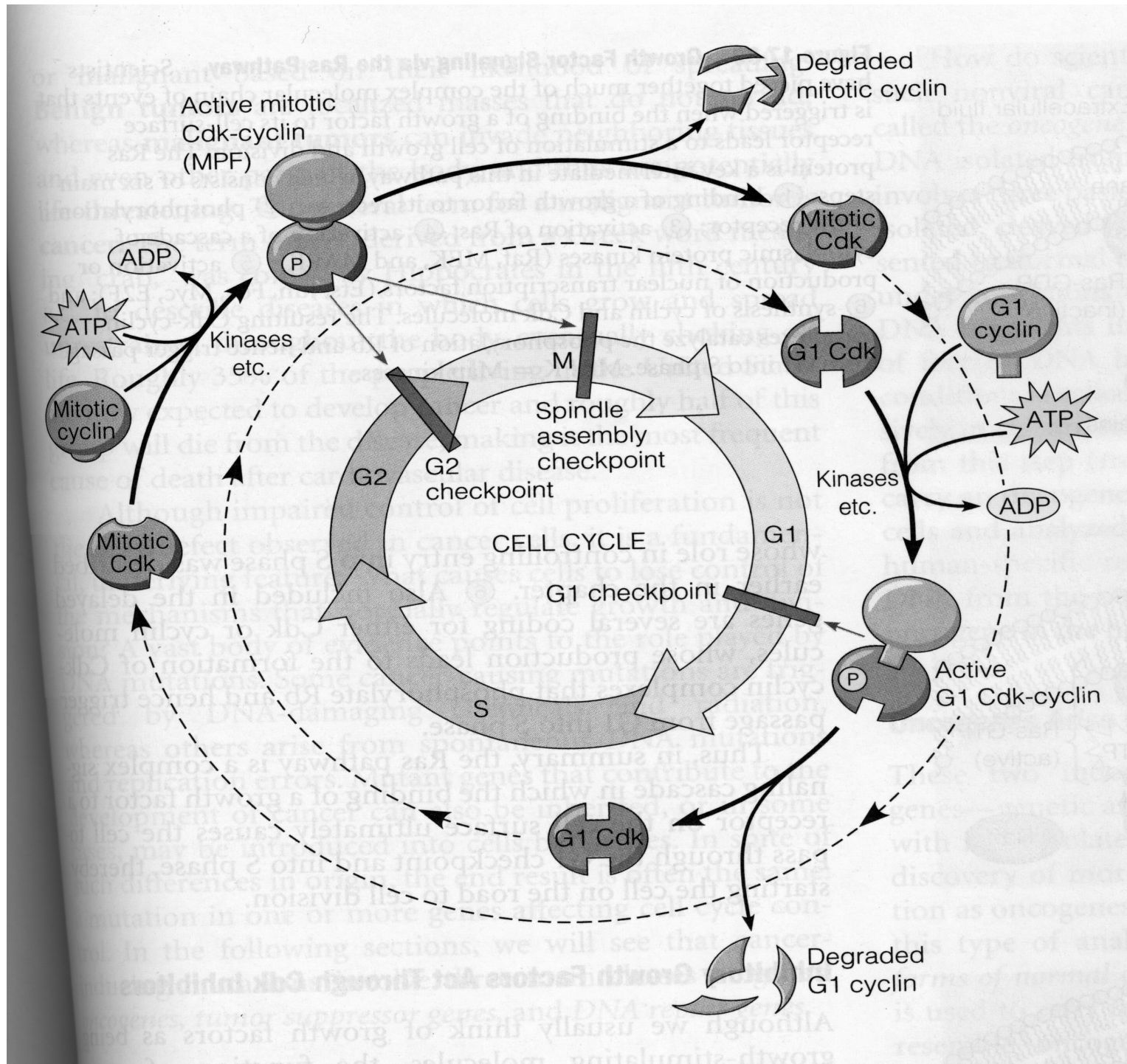
- Activation of p53 causes:

- Cell cycle arrest: p53 acts as a transcription factor that activates the gene coding for **p21**, a **Cdk inhibitor** that suppresses the activity of Cdk-cyclin complexes and blocks passage through the G1 checkpoint (cannot phosphorylate Rb protein)
- Also, p53 activates enzymes involved in DNA repair
- Cell death: if DNA damage cannot be repaired p53 activates genes that trigger **apoptosis**. Characterized by changes in mitochondrial membrane permeability and release of cytochrome c into cytoplasm. **Caspases** (proteases) degrade cells structural proteins leading to disassembly of the dying cell (leads to cell shrinkage, collapse of cytoskeleton, nuclear envelope breakdown and chromatin condensation and degradation of DNA).
- Individuals who inherit only one functional copy of the p53 gene (Li-Fraumeni syndrome) increase likelihood of cancers developing
 - ❖ Most cancers due to spontaneous mutations due to environmental factors that cause point mutations of p53

- Summary of Cell Cycle

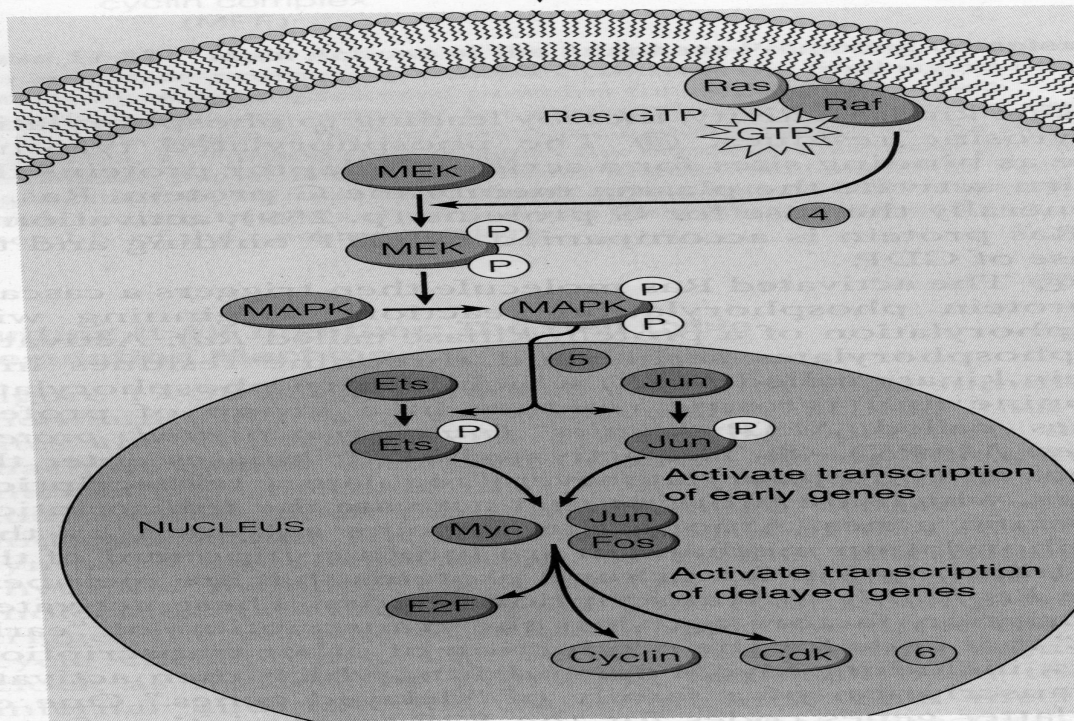
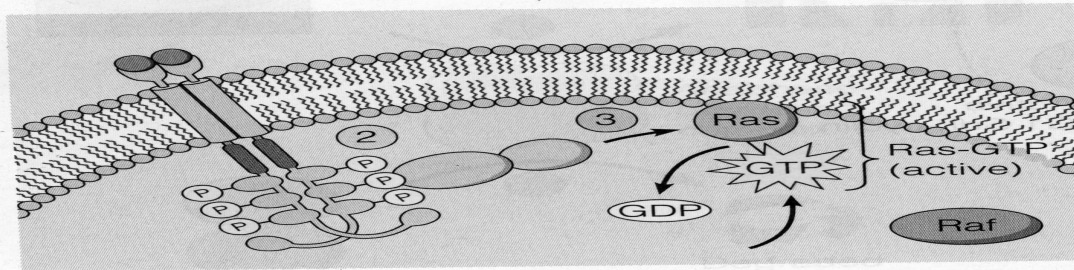
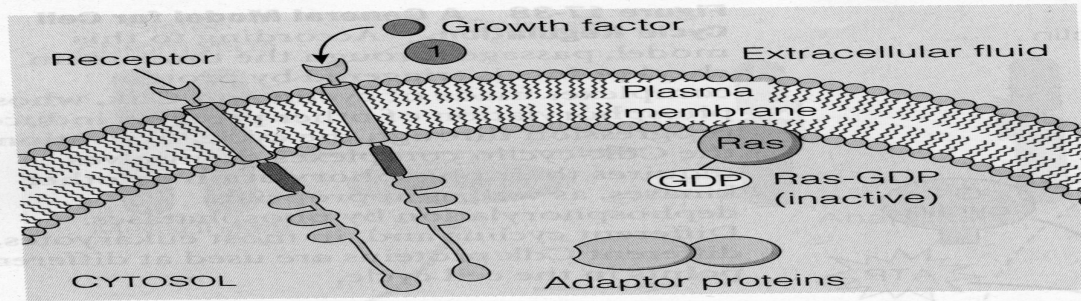
- 2 mechanisms

- Autonomous clock that goes through a fixed cycle over and over again by:
 - Synthesis and degradation of cyclins. These bind to Cdk creating various complexes that trigger passage of cells through the 3 checkpoints
 - Adjustment of clock as needed: molecules that regulate activity of Cdk-cyclin complexes (kinases and phosphatases)



Growth Control and Cancer

- Activation of ras pathway:
 - Tyrosine kinase
 - Binding of membrane receptors that activated ras
 - Binding by growth factors
 - Platelet-derived growth factor (PDGF)
 - Epidermal growth factor (EGF)
- **GF binds to memb receptor → activ TK activity → phos tyrosines activate G protein ras (GTP binding and release of GDP) → Raf → MEK → MAP kinases → activates transcription factors jun, fos, AP1, myc → formation of Cdk-cyclin complexes**



Fl
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Inhibitory Growth Factors

- Transforming growth factor-beta (TGF β)
 - Can both promote and inhibit, depending upon cell type and receptor