Peter Pristas

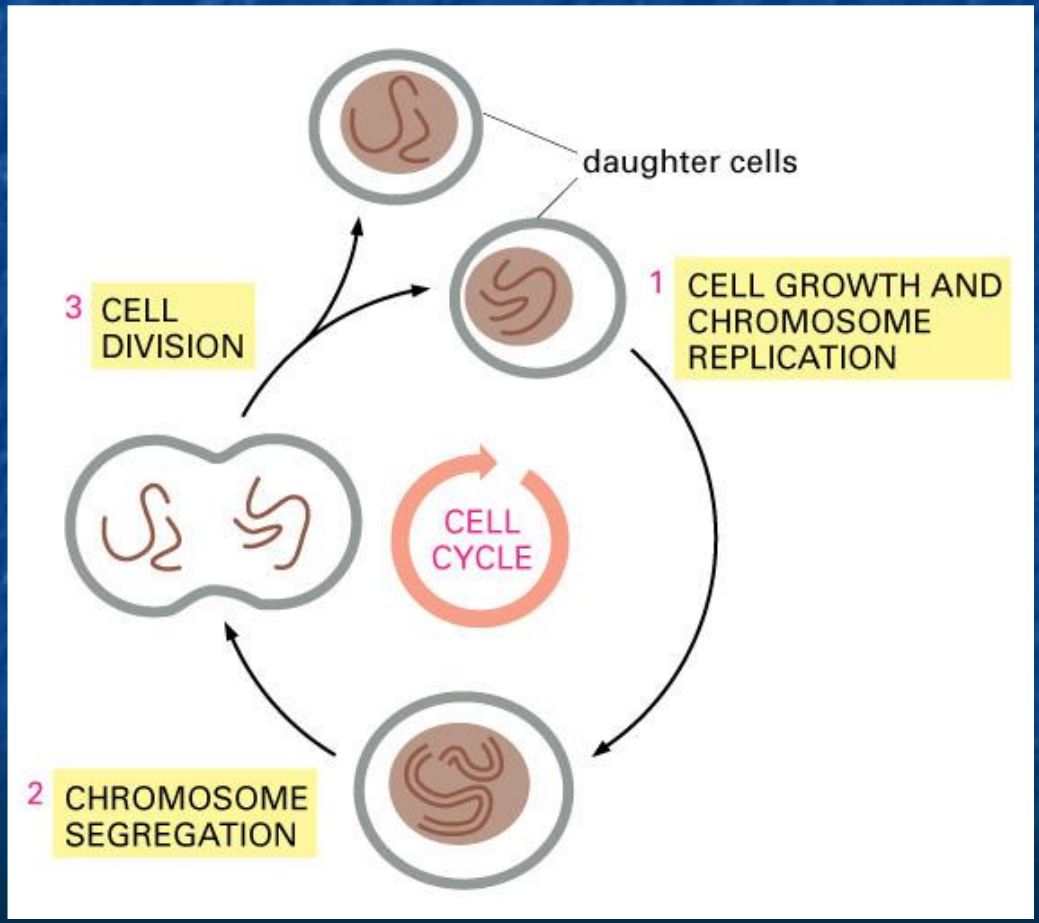
Molecular Biology

Cell cycle regulation

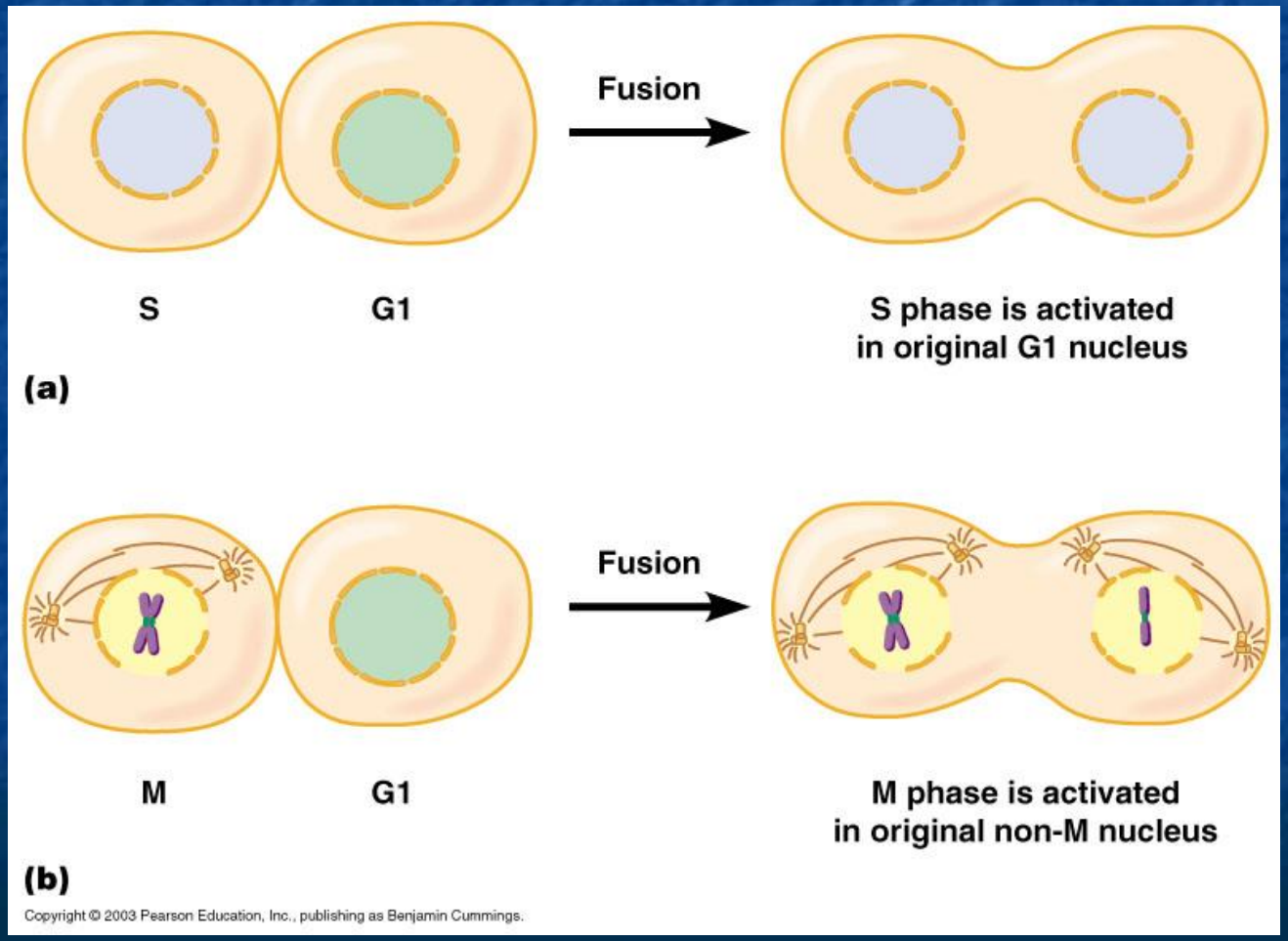
Coordination of cell division

- A multicellular organism needs to coordinate cell division across different tissues & organs
  - critical for normal growth, development & maintenance
    - coordinate timing of cell division
    - coordinate rates of cell division
    - not all cells can have the same cell cycle

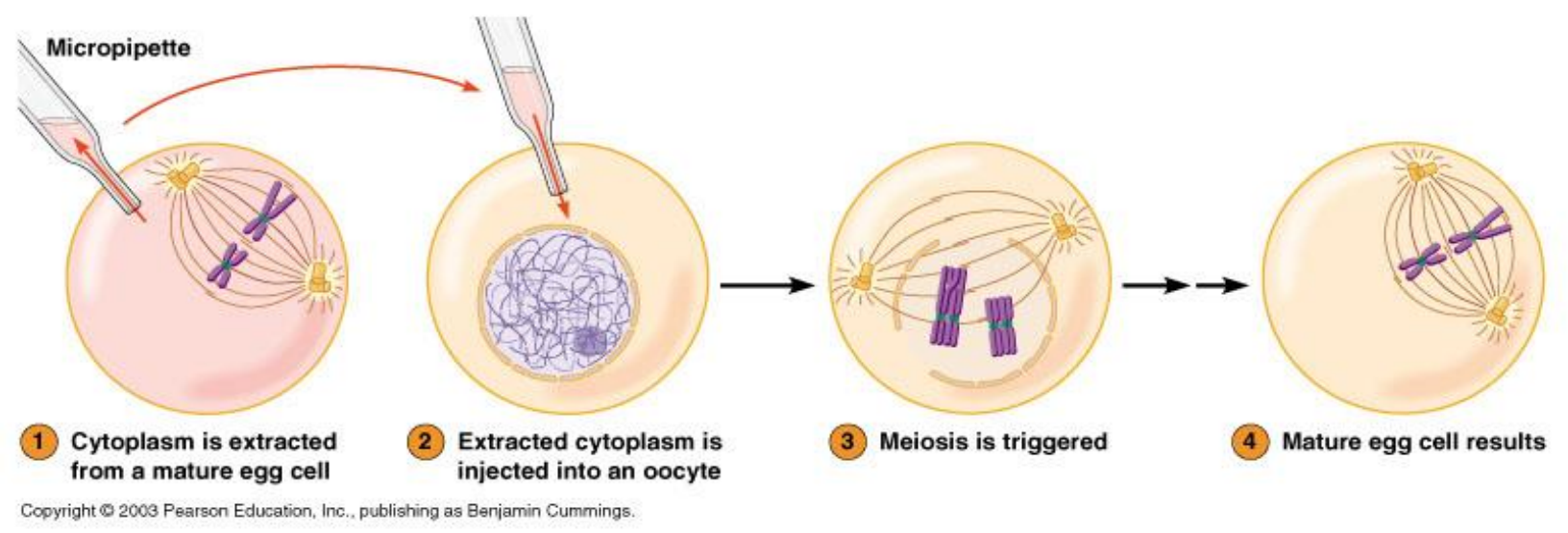
The cell cycle: cells duplicate their contents and divide



Evidence that Cytoplasmic Signals Control the Cell Cycle



Evidence for a Maturation (or Mitosis) Promoting Factor (MPF) – Masui et al. 1971

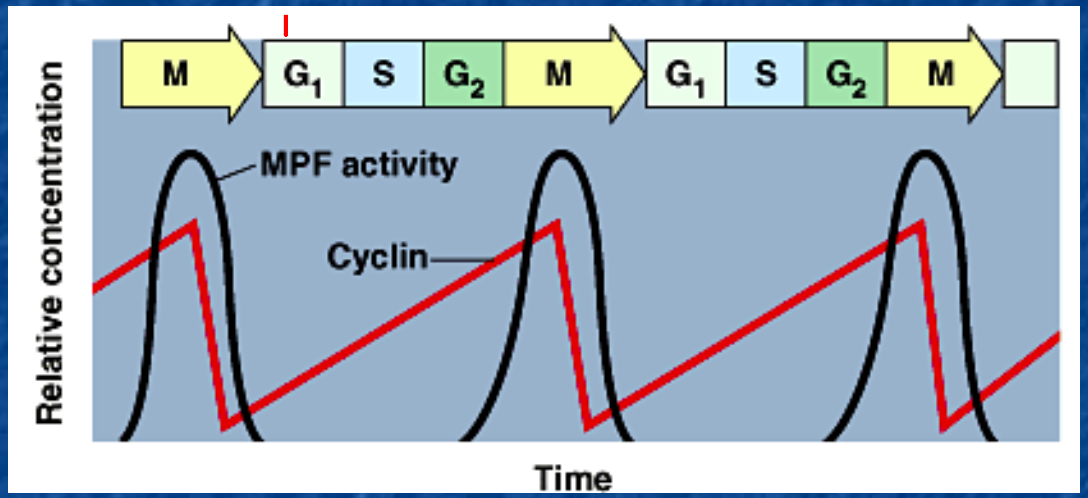


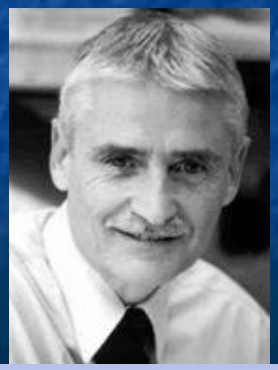
Cell Division Cycle (cdc) Mutants in Yeast (late 1980's)

- Hartwell (Nobel Prize, 2001) et al., working with budding yeast *S. cerevisiae*
  - Found temperature-sensitive mutants stuck in some point of cell cycle
- Nurse (Nobel Prize, 2001) et al., working with fission yeast *S. pombe*
  - Found gene they called cdc2, essential for passing G2 checkpoint
  - cdc2 turned out to be a new protein kinase - cyclin dependant kinase (Cdk) - with counterparts in all eukaryotic cells.

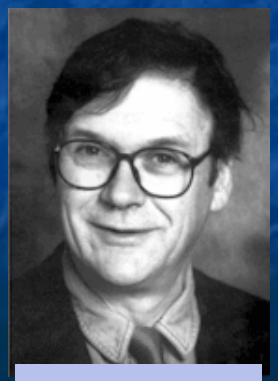
Cyclins & Cdks

- Interaction of Cdk's & different cyclins triggers the stages of the cell cycle





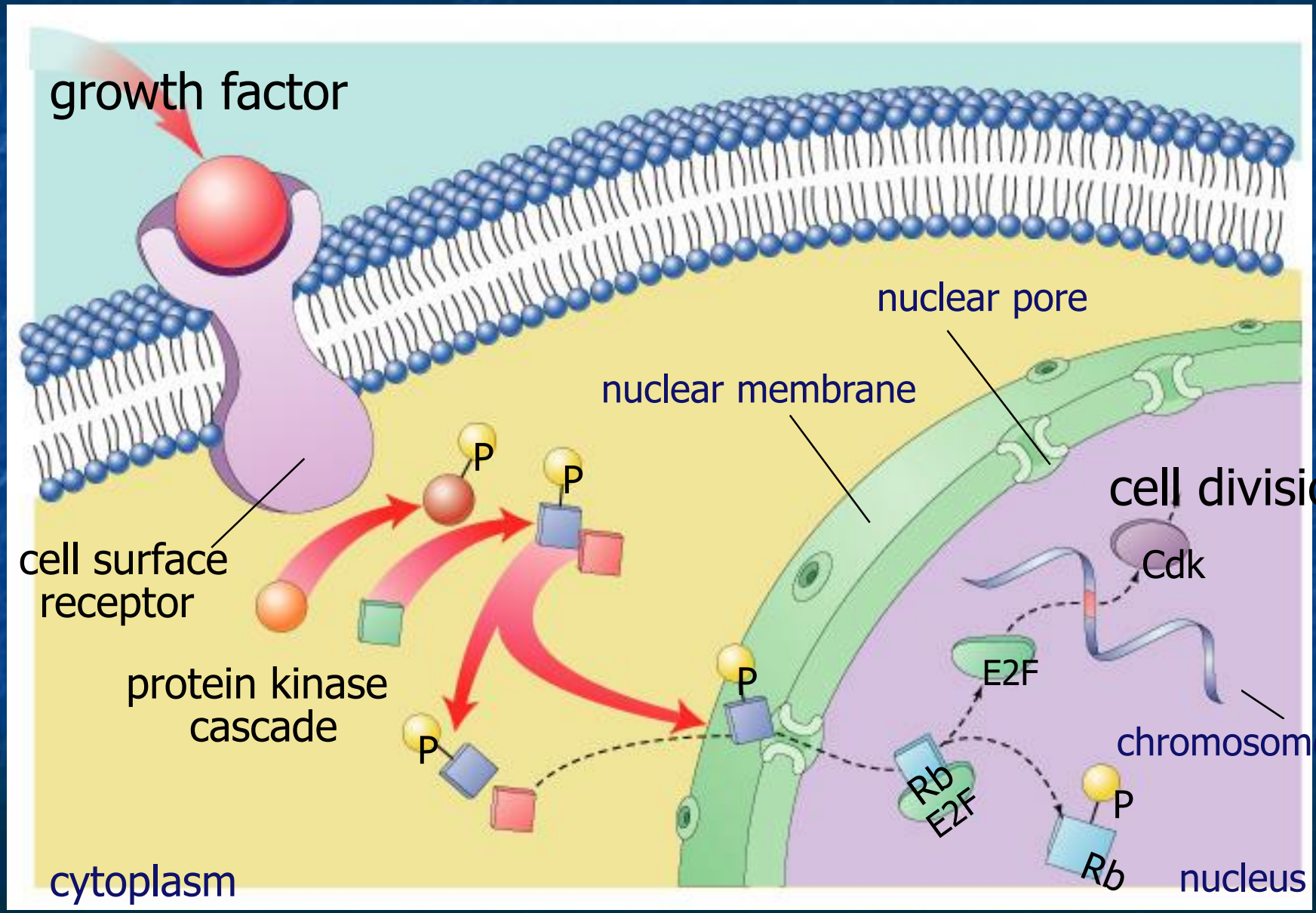
Leland H. Hartwell
checkpoints



Tim Hunt
Cdks

Sir Paul Nurse
cyclins

Growth factor signals



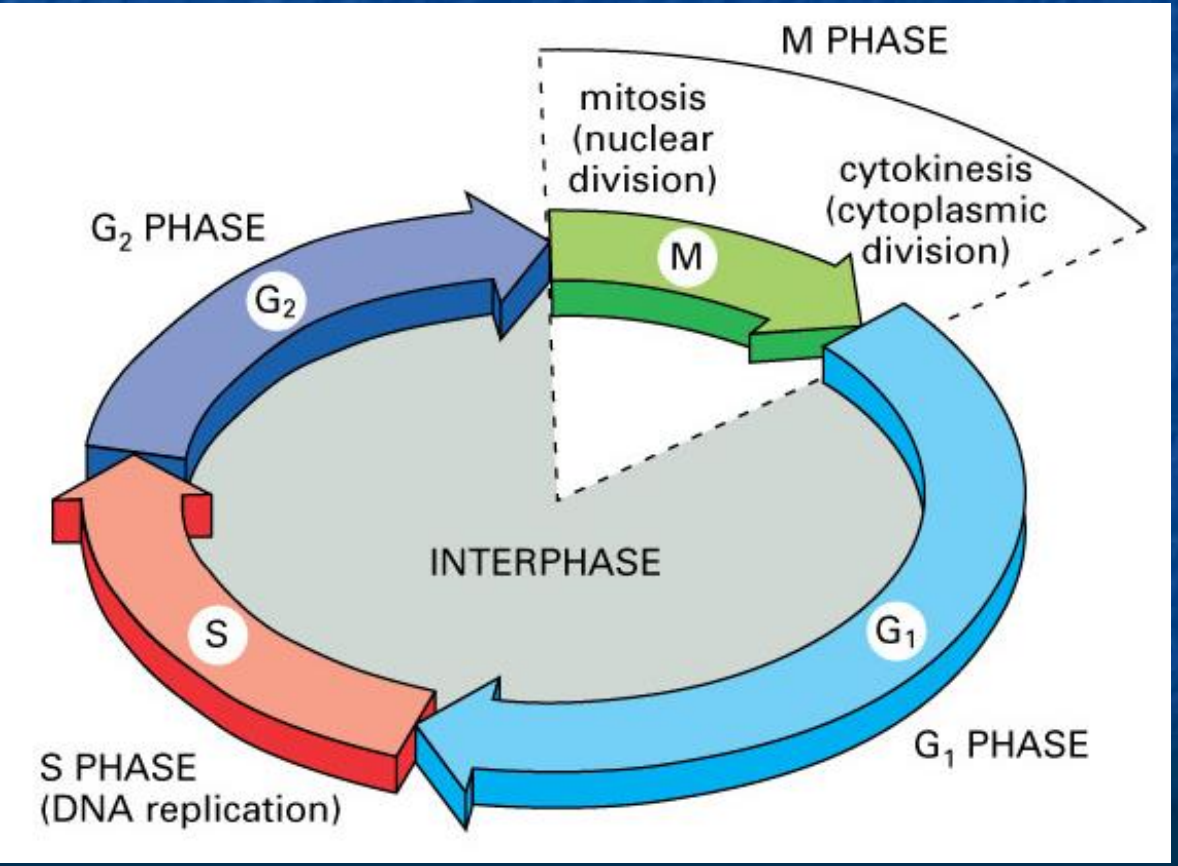
Erythropoietin (EPO):

- **Erythropoietin (EPO): A hormone produced by the kidney that promotes the formation of red blood cells in the bone marrow.**
- **The kidney cells that make EPO are specialized and are sensitive to low oxygen levels in the blood. These cells release EPO when the oxygen level is low in the kidney. EPO then stimulates the bone marrow to produce more red cells and thereby increase the oxygen-carrying capacity of the blood.**

Frequency of cell division

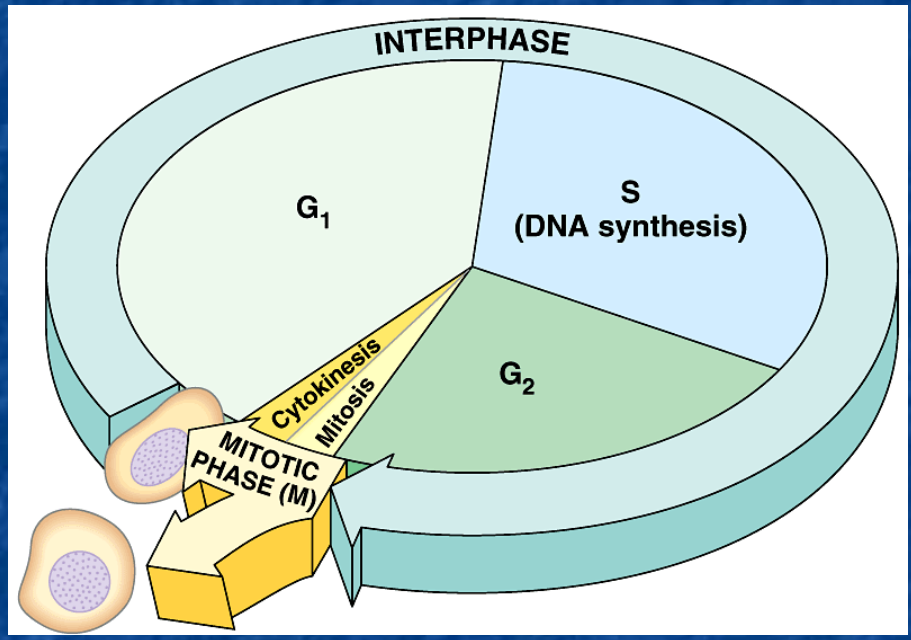
- Frequency of cell division varies by cell type
  - embryo
    - cell cycle < 20 minute
  - skin cells
    - divide frequently throughout life
    - 12-24 hours cycle
  - liver cells
    - retain ability to divide, but keep it in reserve
    - divide once every year or two
  - mature nerve cells & muscle cells
    - do not divide at all after maturity
    - permanently in G_0

The cell cycle may be divided into 4 phases



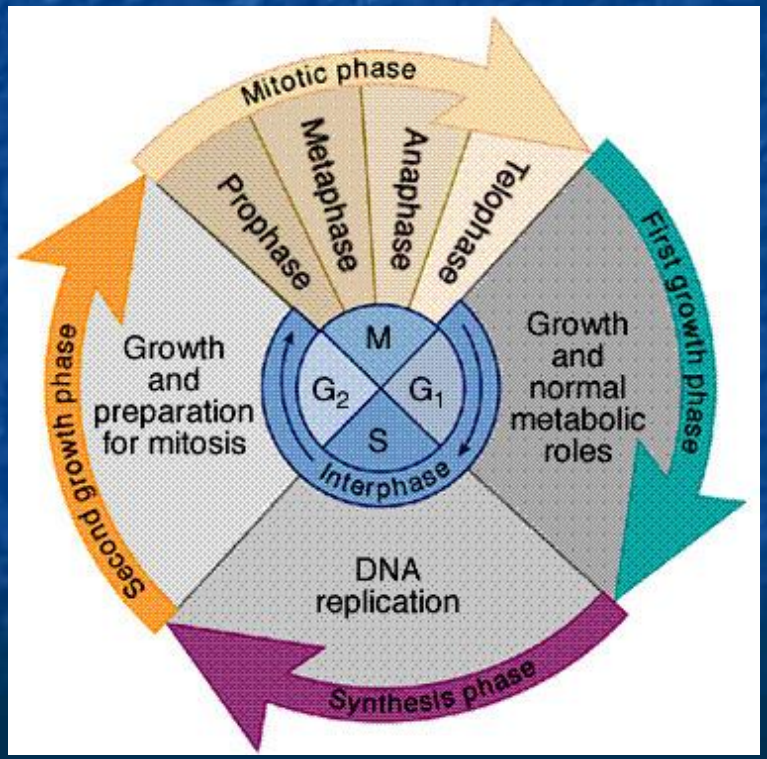
Interphase

- 90% of cell life cycle
  - cell doing its "everyday job"
    - produce RNA, synthesize proteins/enzymes
  - prepares for duplication if triggered



Interphase

- Divided into 3 phases:
  - G_1 = 1st Gap (Growth)
    - cell doing its "everyday job"
    - cell grows
  - S = DNA Synthesis
    - copies chromosomes
    - Duplicates centrioles
  - G_2 = 2nd Gap (Growth)
    - prepares for division
    - cell grows (more)
    - produces organelles, proteins, membranes



Progression of the cell cycle is regulated by feedback from intracellular events

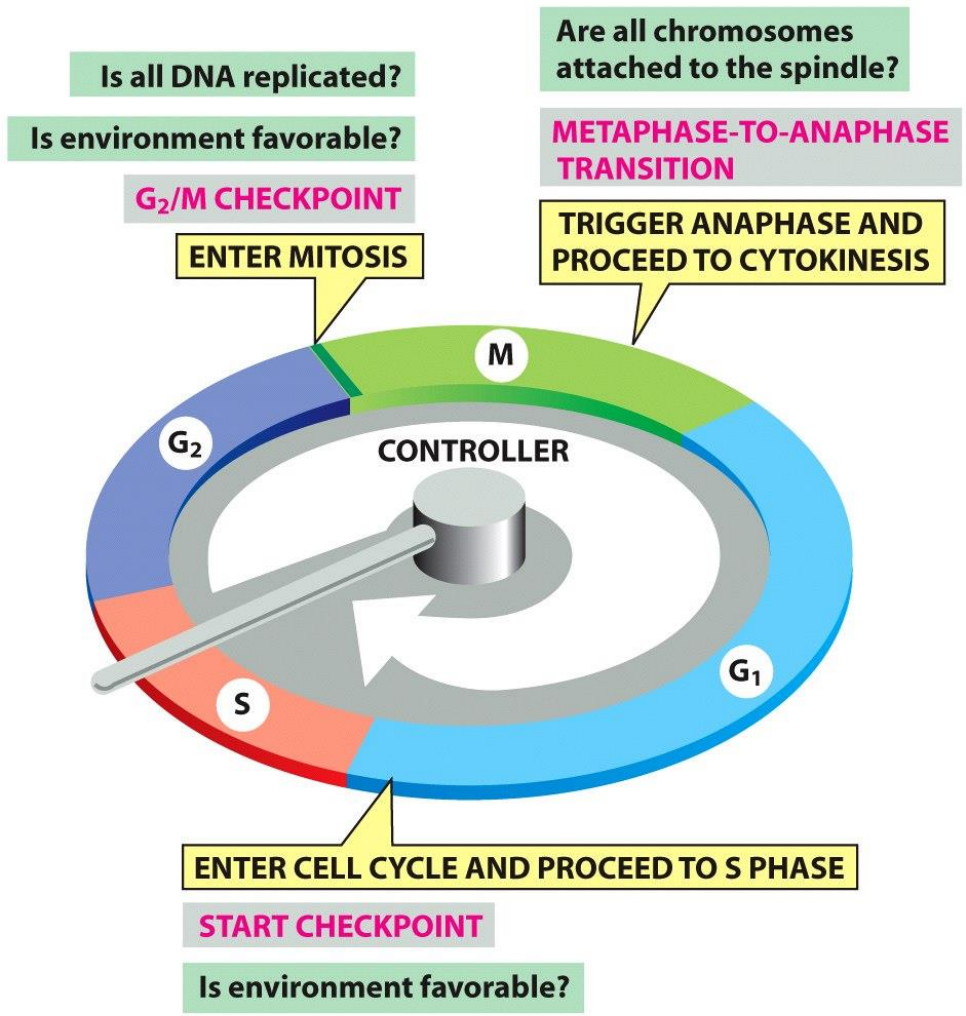
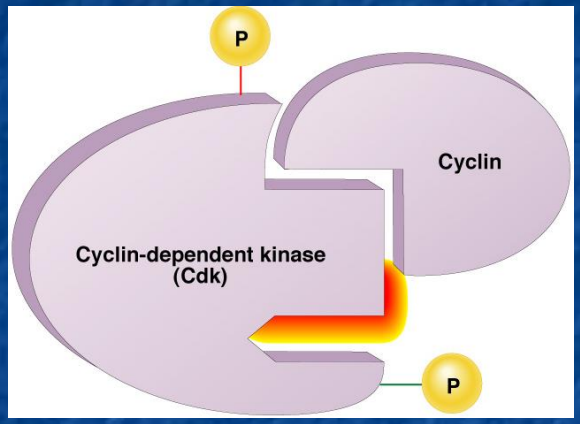


Figure 17-14 Molecular Biology of the Cell 5/e (© Garland Science 2008)

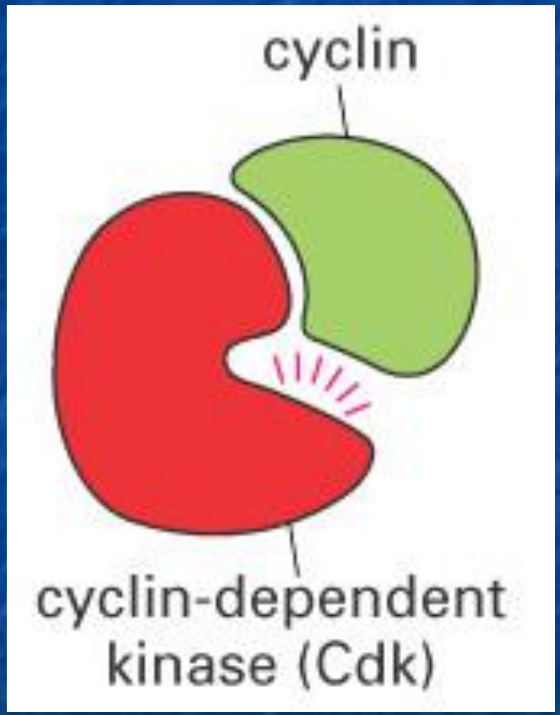
Cell cycle signals

- Cell cycle controls
  - cyclins
    - regulatory proteins
    - levels cycle in the cell
  - Cdks
    - cyclin-dependent kinases
    - phosphorylates cellular proteins
      - activates or inactivates proteins
  - Cdk-cyclin complex
    - triggers passage through different stages of cell cycle

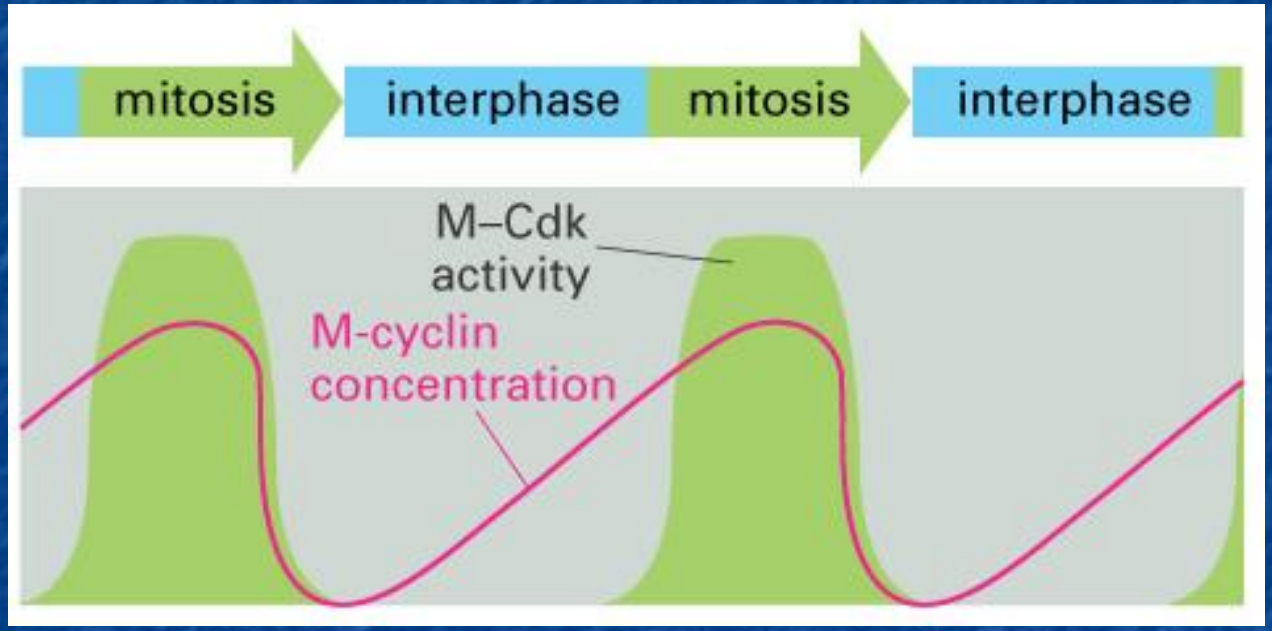


Cyclin-dependent protein kinases drive progression through the cell cycle

- Cyclin-dependent kinases (Cdks) are inactive unless bound to cyclins
- Active complex phosphorylates downstream targets
- Cyclin helps to direct Cdks to the target proteins

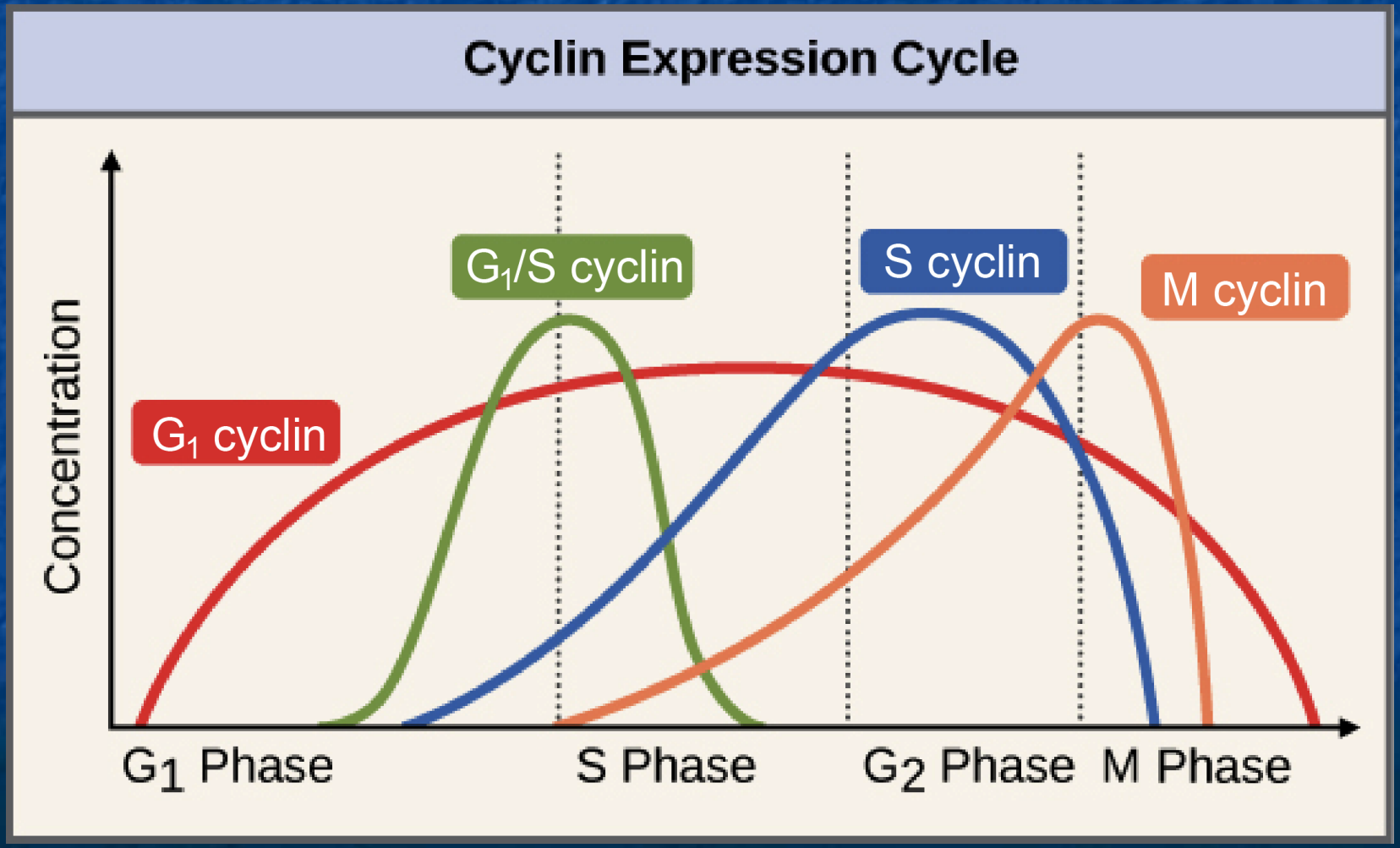


Cellular levels of (mitotic) M-cyclin rises and falls during the cell cycle

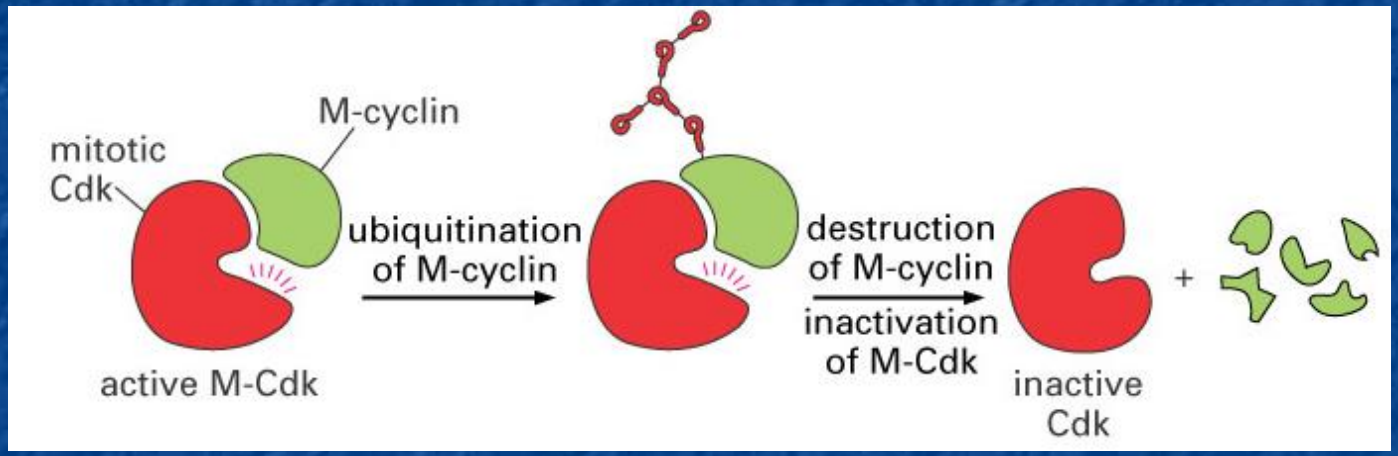


- M-cyclin levels are low during interphase but gradually increases to a peak level during mitosis
- M-cdk activity is, likewise, low in interphase but increases in mitosis

Cellular levels of cyclins during the cell cycle

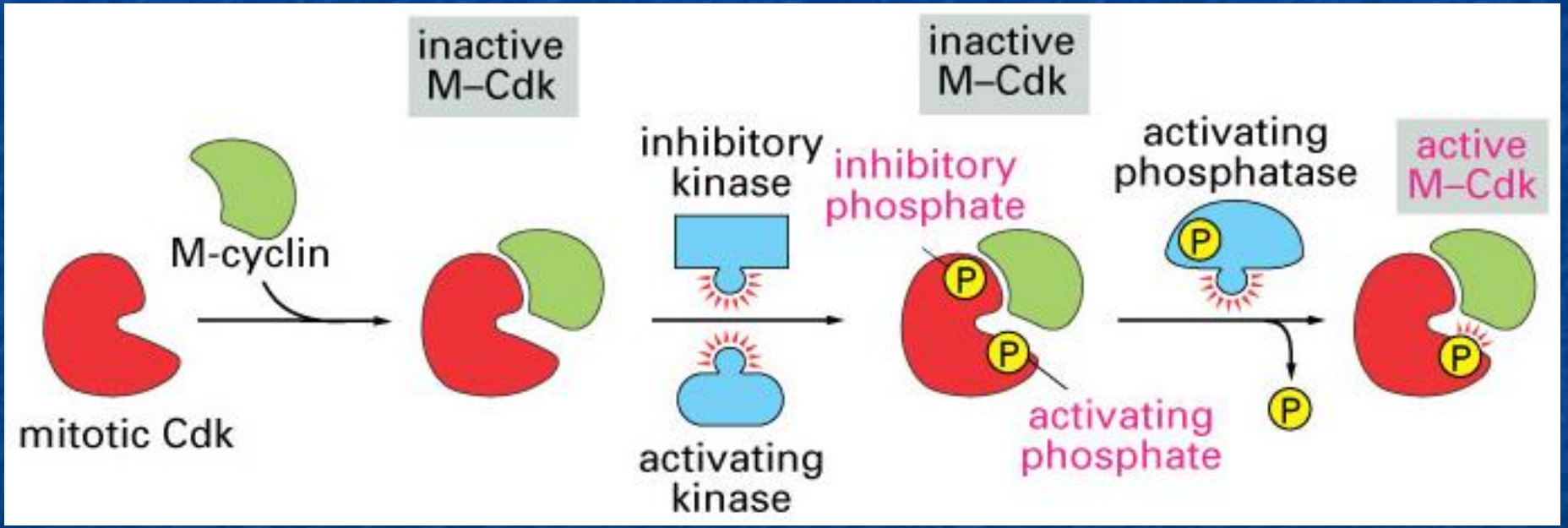


The abundance of cyclins (and the activity of Cdks) is regulated by protein degradation

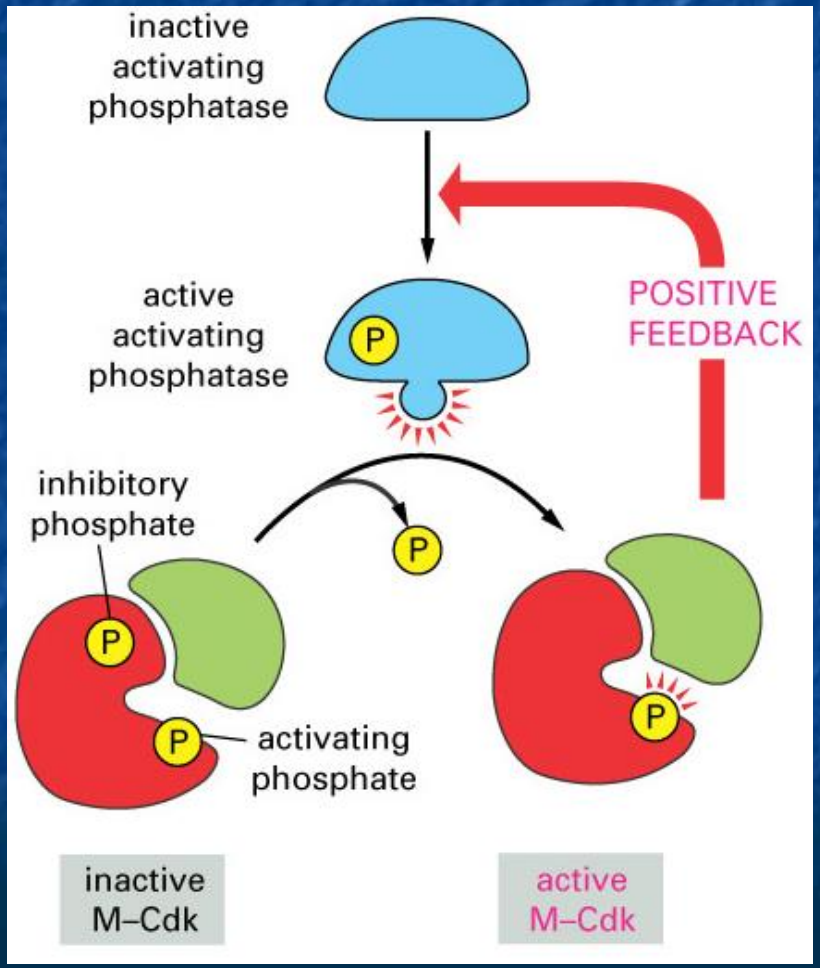


- M-cyclin becomes covalently modified by addition of multiple copies of ubiquitin at the end of mitosis
- Ubiqutination is mediated by the anaphase promoting complex (APC)
- Ubiquitination marks cyclins for destruction by large proteolytic machines called proteasome

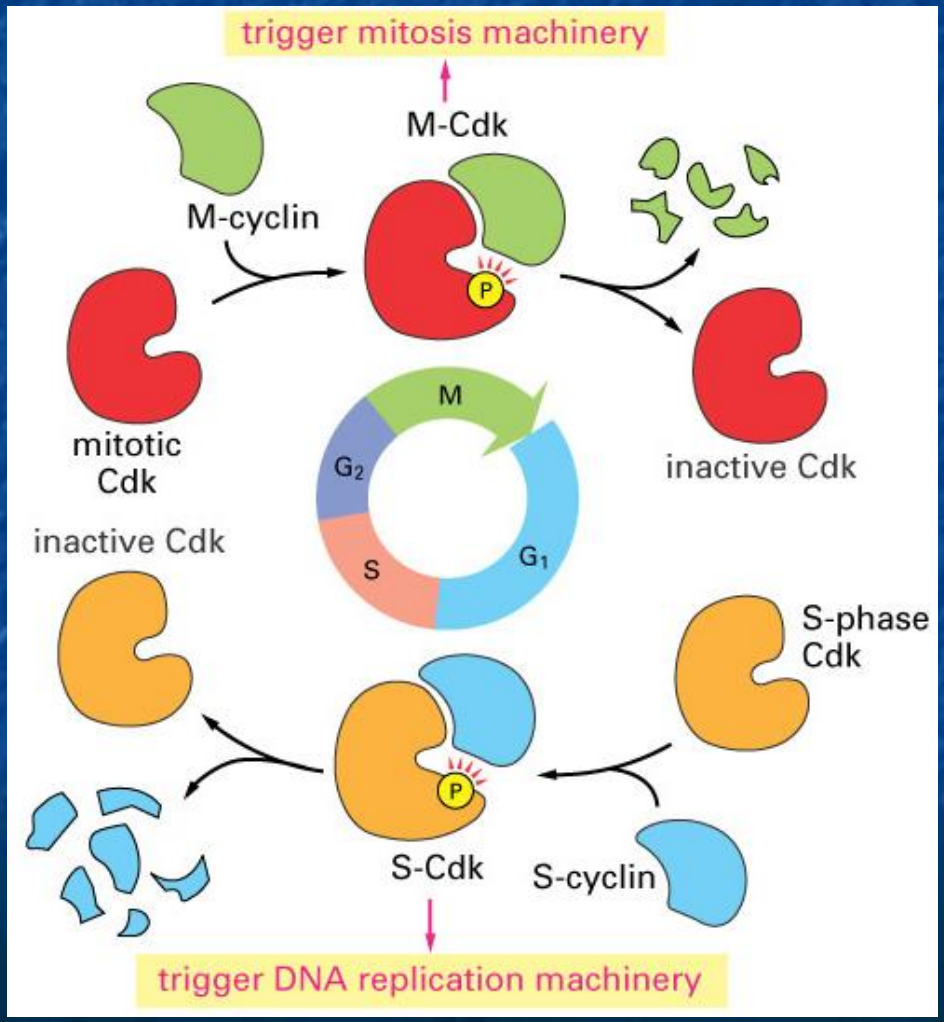
Cdks are also regulated by cycles of phosphorylation and dephosphorylation



Cdk activates itself indirectly via a positive feedback loop



Distinct cyclins partner with distinct Cdks to trigger different events of the cell cycle



S-Cdk triggers DNA replication - its destruction ensures this happens once per cell cycle

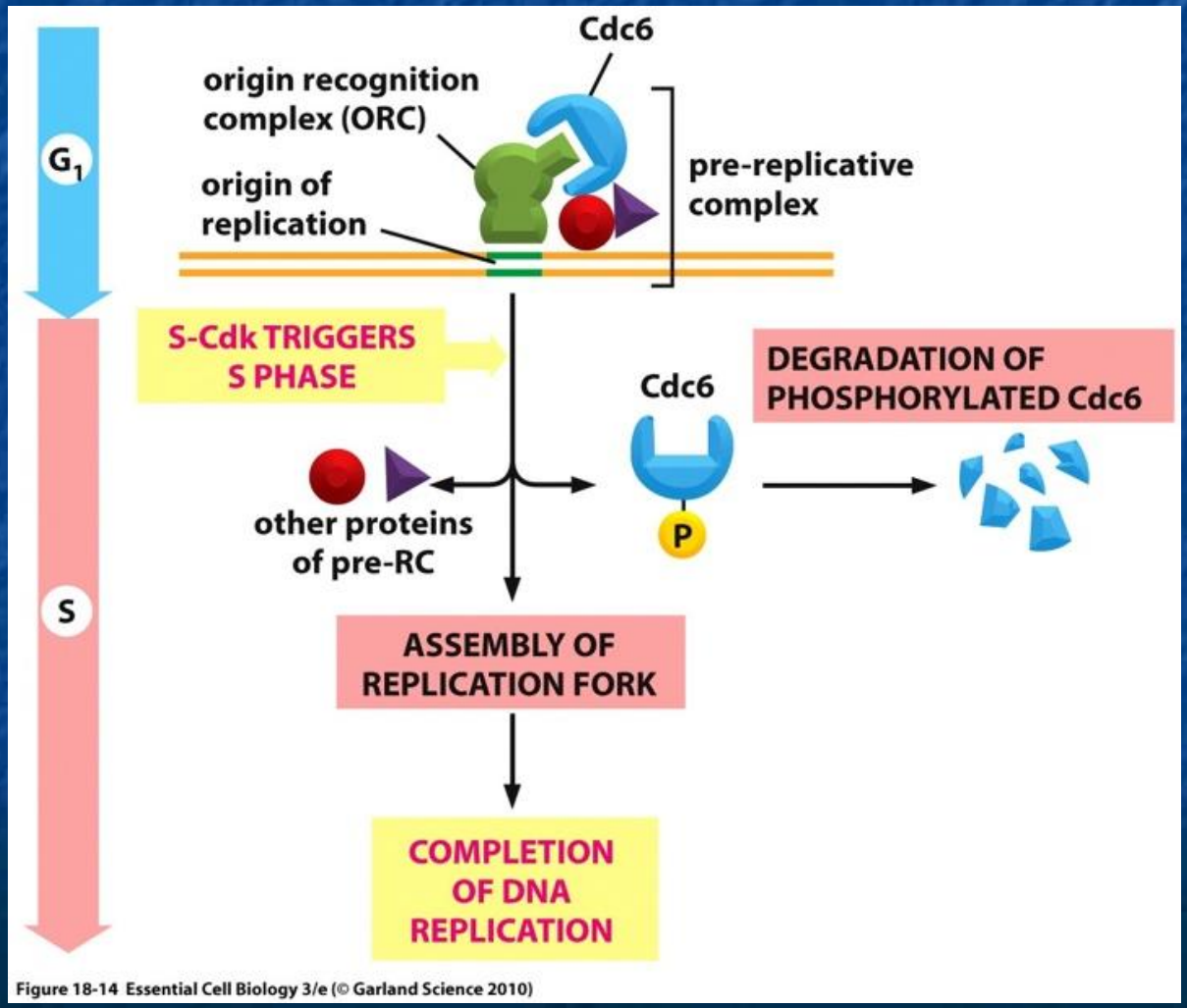


Figure 18-14 Essential Cell Biology 3/e (© Garland Science 2010)

Checkpoints ensure the cell cycle proceeds without errors

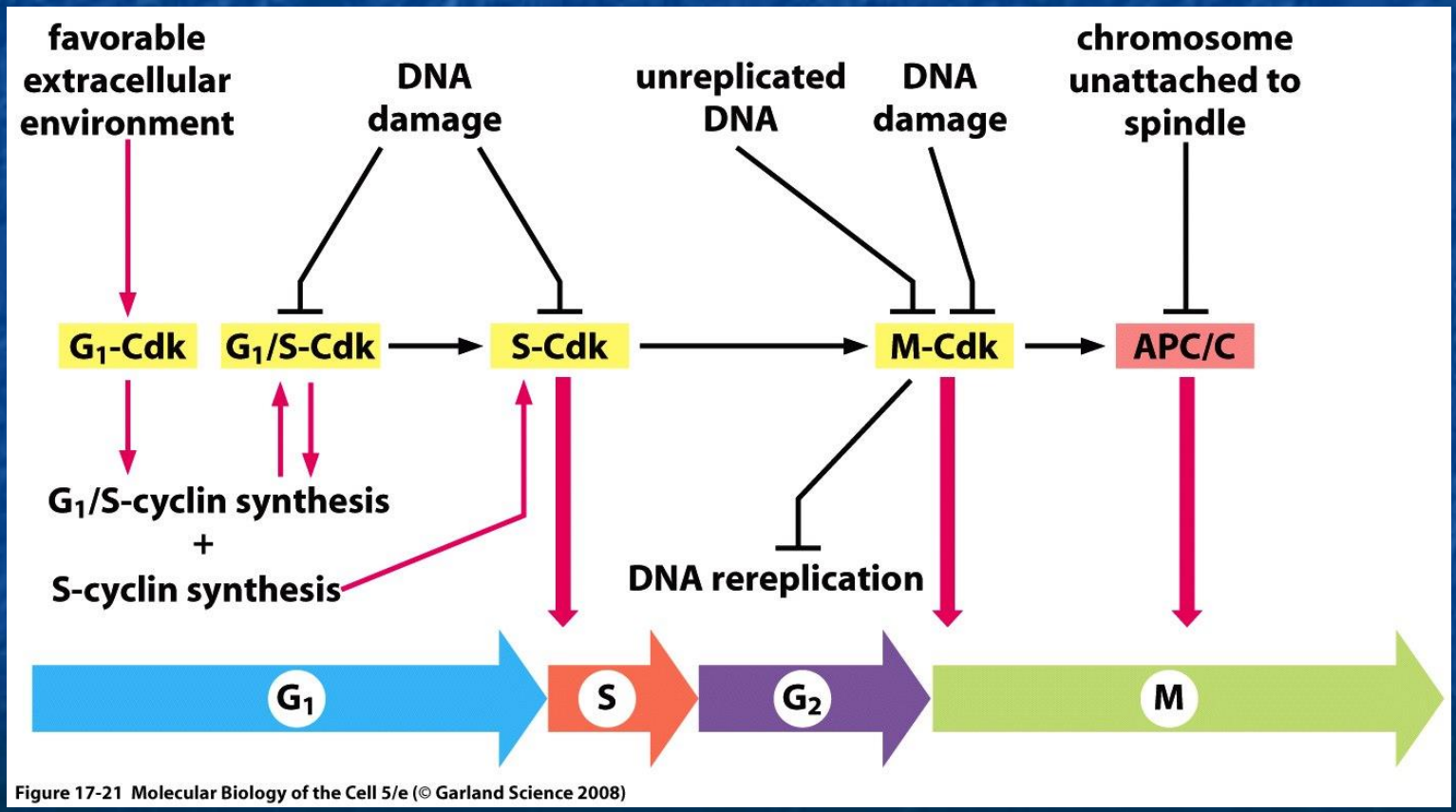


Figure 17-21 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Checkpoints - pRB- retinoblastoma protein

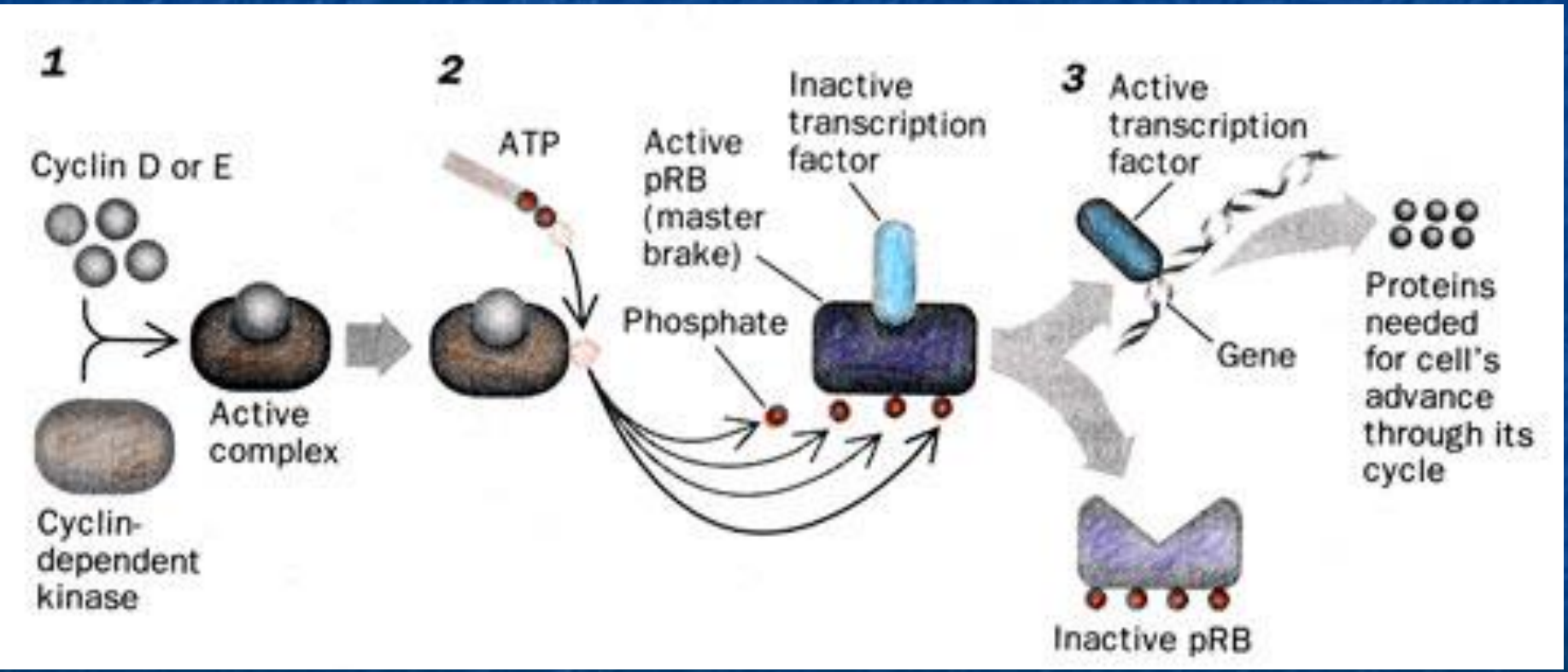
named so because in retinoblastoma cancer both alleles of the gene are mutated so no protein is produced

pRb prevents the cell from dividing or progressing through the cell cycle when DNA is damaged.

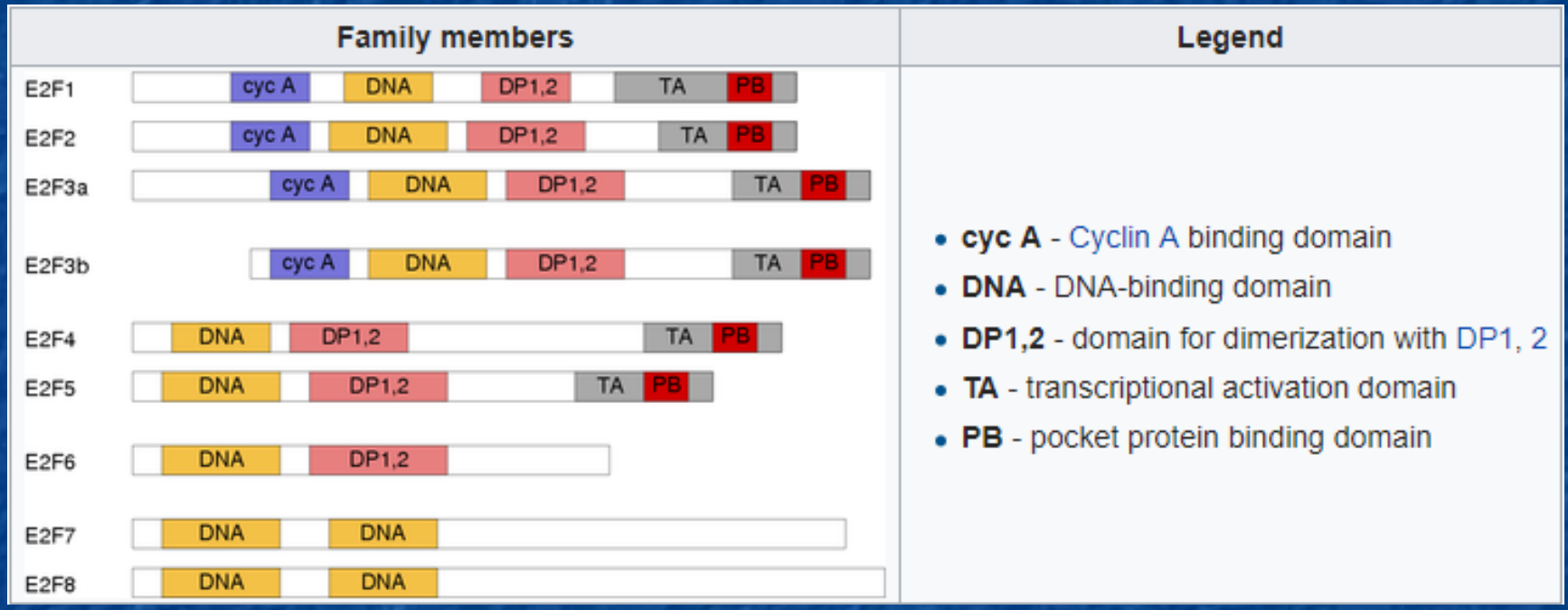
Control occurs at S (DNA synthesis phase) because pRb binds and inhibits transcription factors of the E2F family

When pRb is ineffective at this role, mutated cells can continue to divide and may become cancerous.

Checkpoints - pRB- retinoblastoma protein

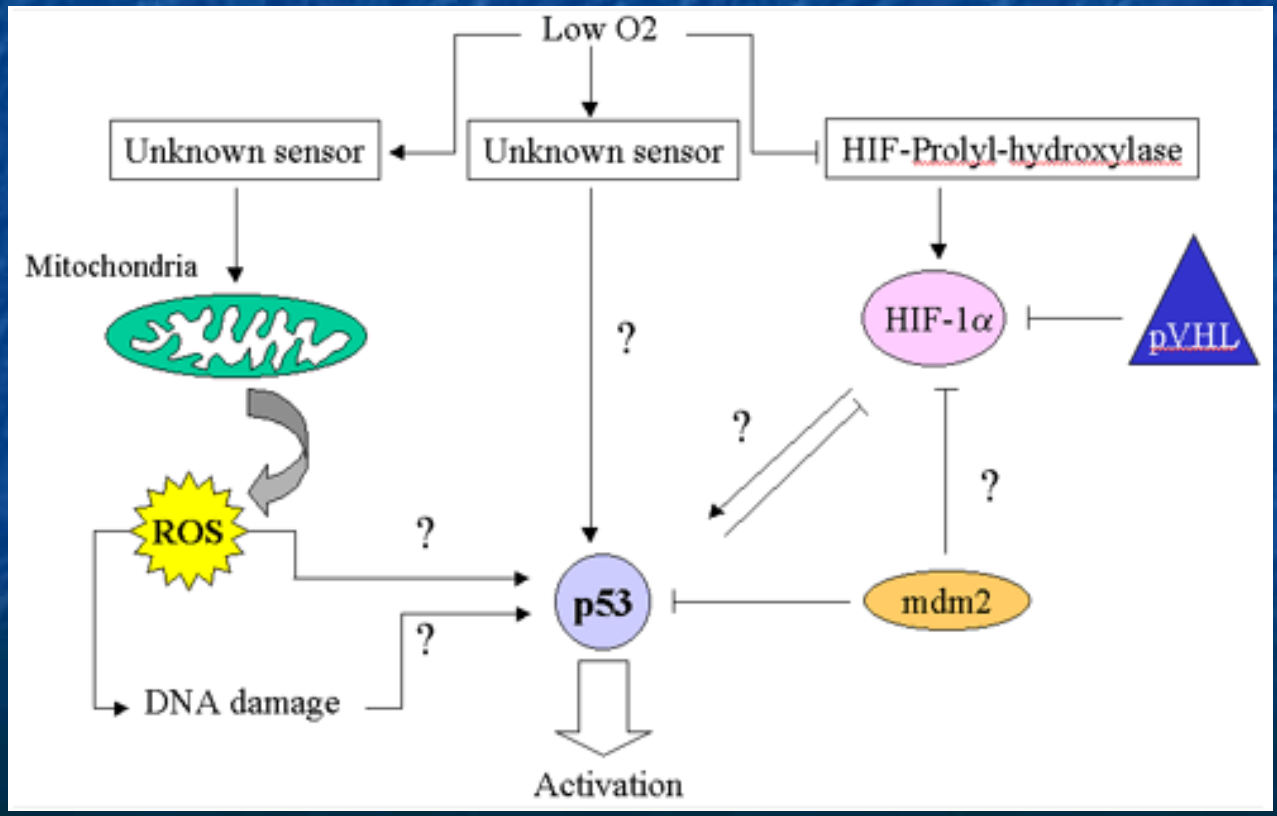


Checkpoints - pRB- retinoblastoma protein – E2F factors

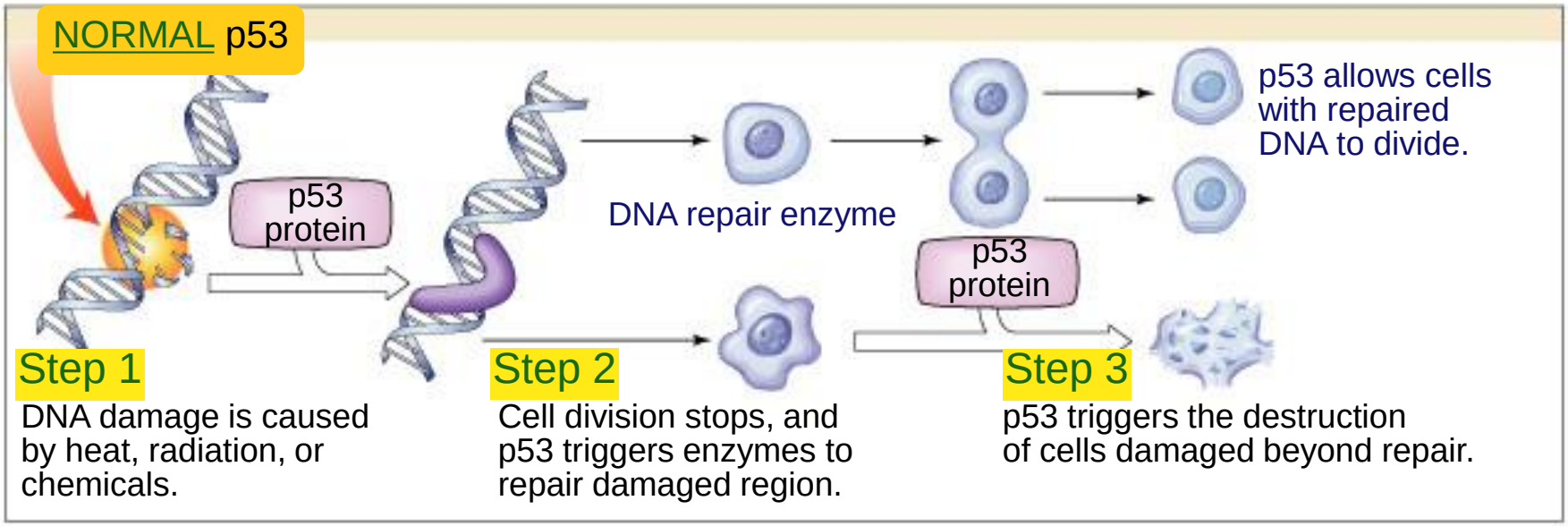


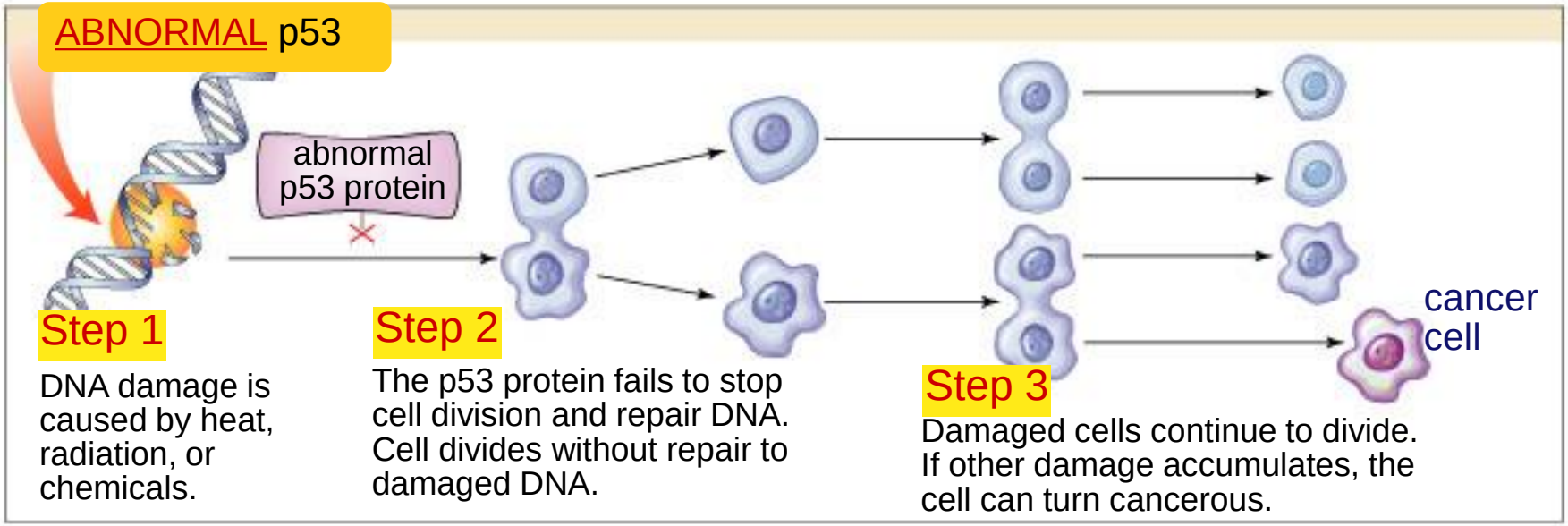
Checkpoints – p53 protein

Hypoxia, cellular exposure to reactive oxygen species, mitochondrial damage, or direct damage of DNA by chemicals or radiation can stimulate p53 actions that halt cell division, activate DNA repair mechanisms, &/or stimulate the cell to undergo apoptosis (programmed cell death). This protects the organism from clonal expansion of mutated cells.



p53 — master regulator gene





Checkpoint: DNA damage arrests the cell cycle in G_1

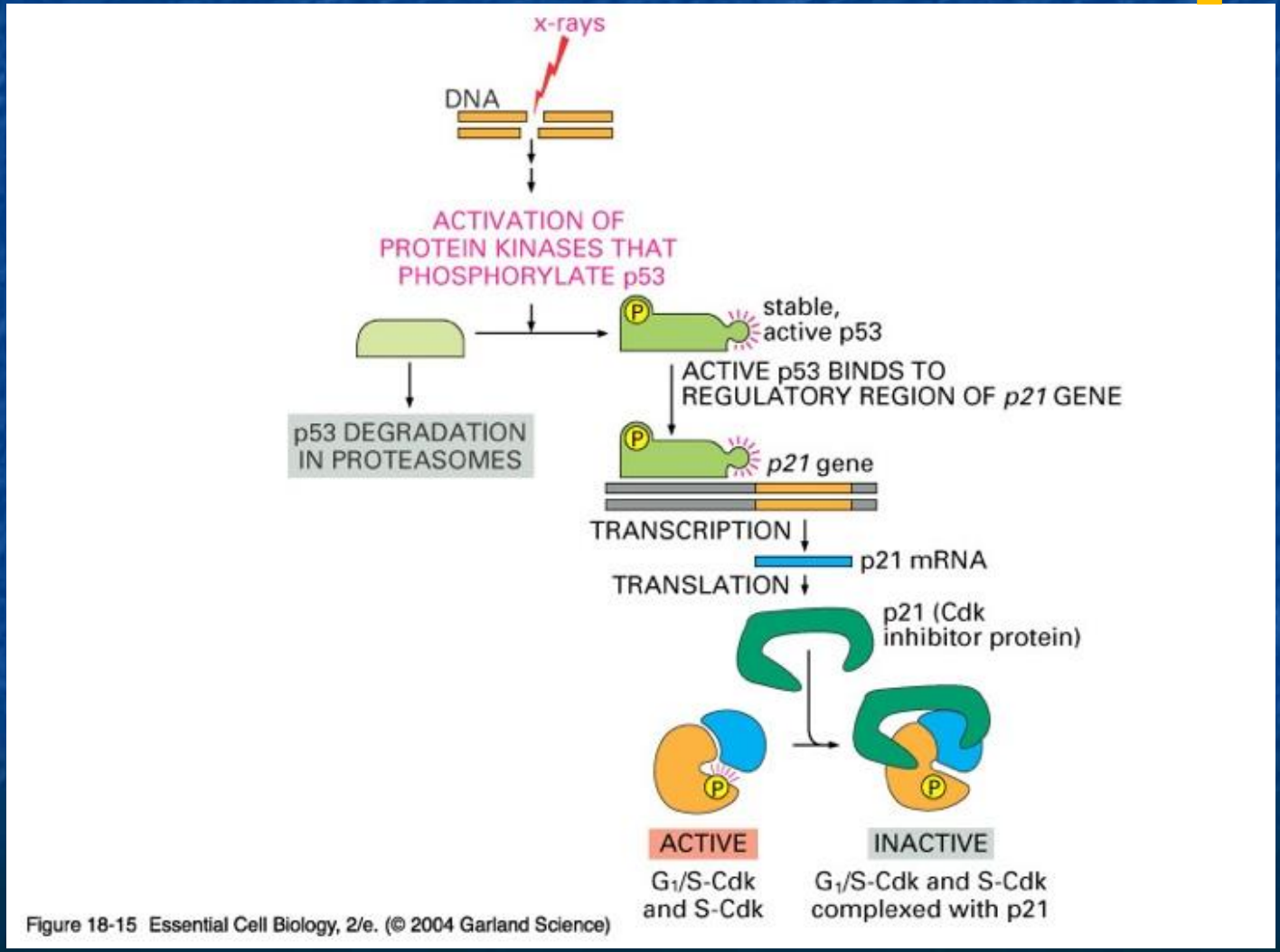


Figure 18-15 Essential Cell Biology, 2/e. (© 2004 Garland Science)

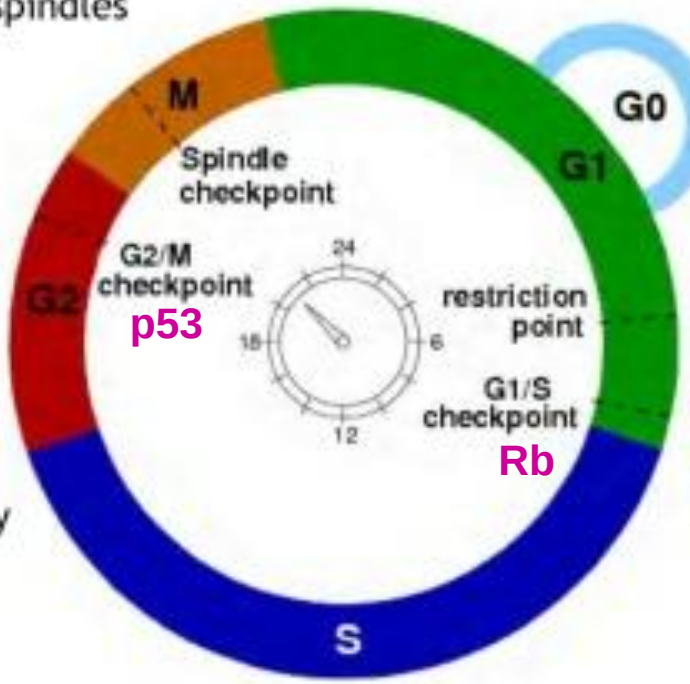
Checkpoint: spindle assembly

- Mitosis must not complete unless all the chromosomes are attached to the mitotic spindle
- Mitotic checkpoint delays metaphase to anaphase transition until all chromosomes are attached
- Prolonged activation of the checkpoint -->cell death
- Mechanism of many anti-cancer drugs

Cell Cycle Checkpoints

M phase – In mitosis chromosomes drawn apart by molecular motors, cell divides. Many cancer drugs like taxol act here freezing the process and causing apoptosis. There is a checkpoint to ensure chromosomes are correctly attached to the spindles before segregation.

G1 is entered when the cell senses growth signals or mitogens. These start the process of cell division.



Cell crosses a restriction point c 8-10 hours into G1 - This is a point of no return: the cell is committed to divide or die.

G2/M - cell arranges and checks chromosomes. There is a major checkpoint here to ascertain that DNA replication has successfully occurred. If not, a normal cell undergoes apoptosis.

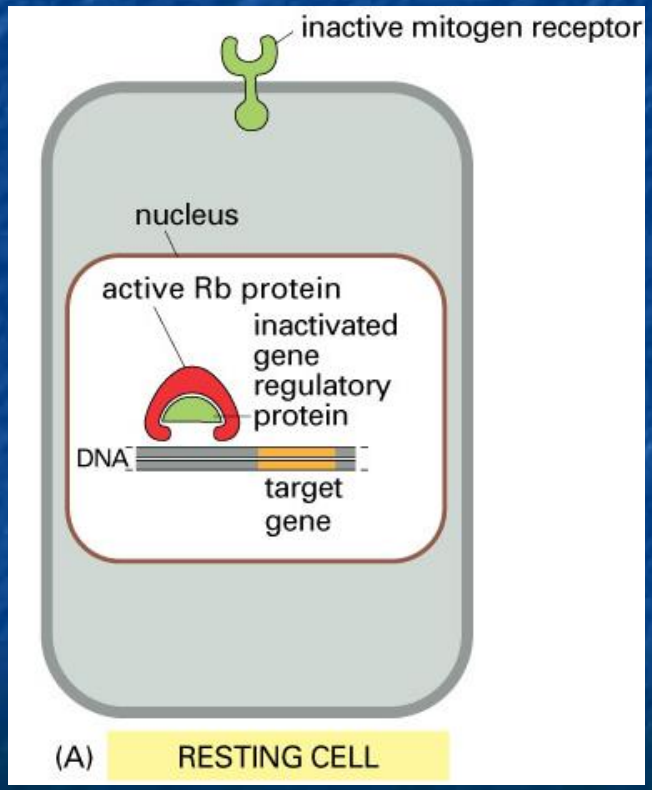
G1/S checkpoint –arrest here for cancer cells leads to apoptosis.

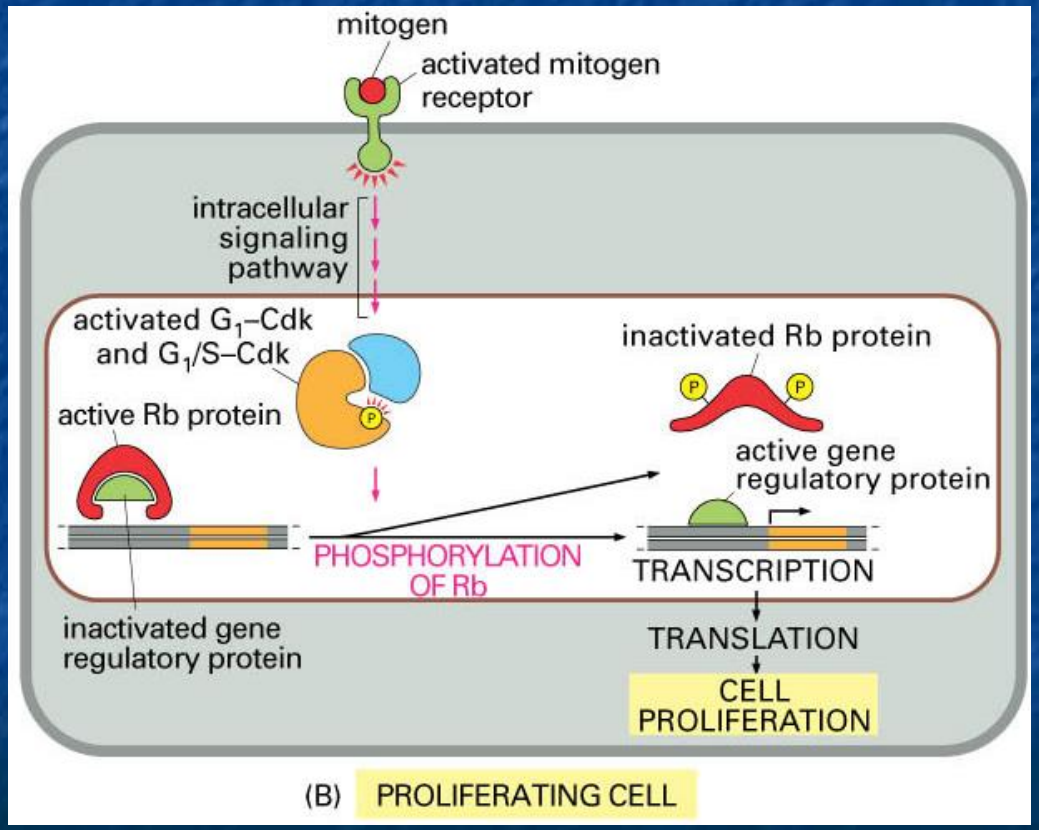
S phase - DNA is synthesised. Many cytotoxic anti-cancer drugs act here to disrupt DNA synthesis.

Animal cells require extracellular signals to divide, grow, and survive

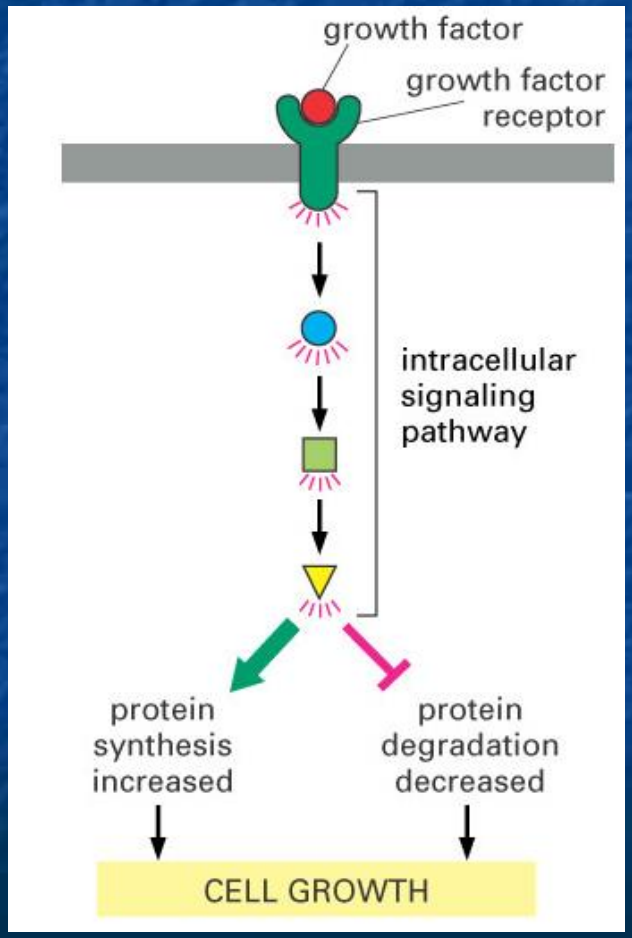
- Mitogens - stimulate cell division by overcoming cell cycle "brake" that leads to G_0
- Growth factors - stimulate growth (increased cell size) by promoting synthesis and inhibiting degradition of macromolecules
- Survival factors - suppress apoptosis

Mitogens stimulate proliferation by inhibiting the Rb protein



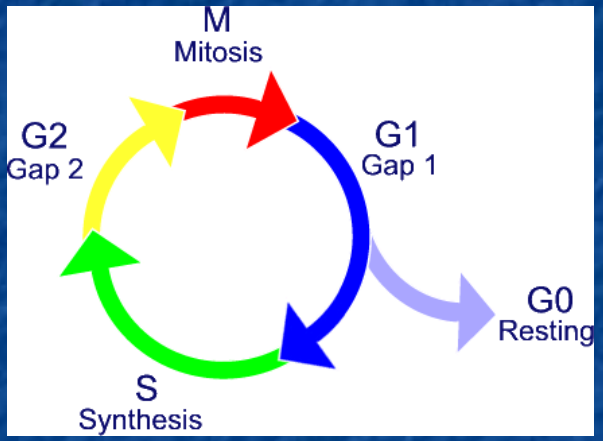


Growth factors increase synthesis & decrease degradation of macromolecules



Cells can withdraw from the cell cycle and dismantle the regulatory machinery

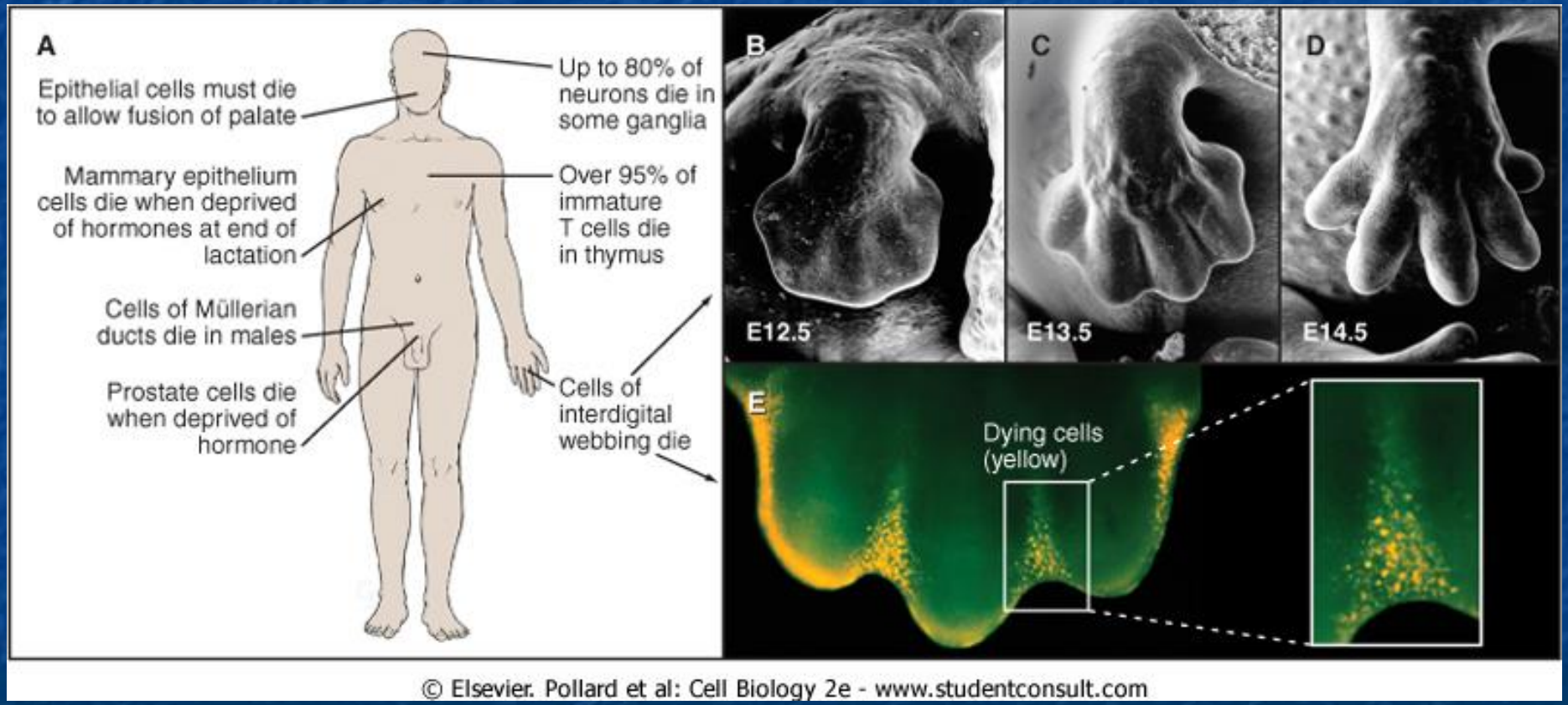
- G_0 is a quiescent state
- Cdks and cyclins disappear
- Some cells enter G_0 temporarily and divide infrequently (I.e. hepatocytes)
- Other differentiated cell types (neurons) spend their life in G_0



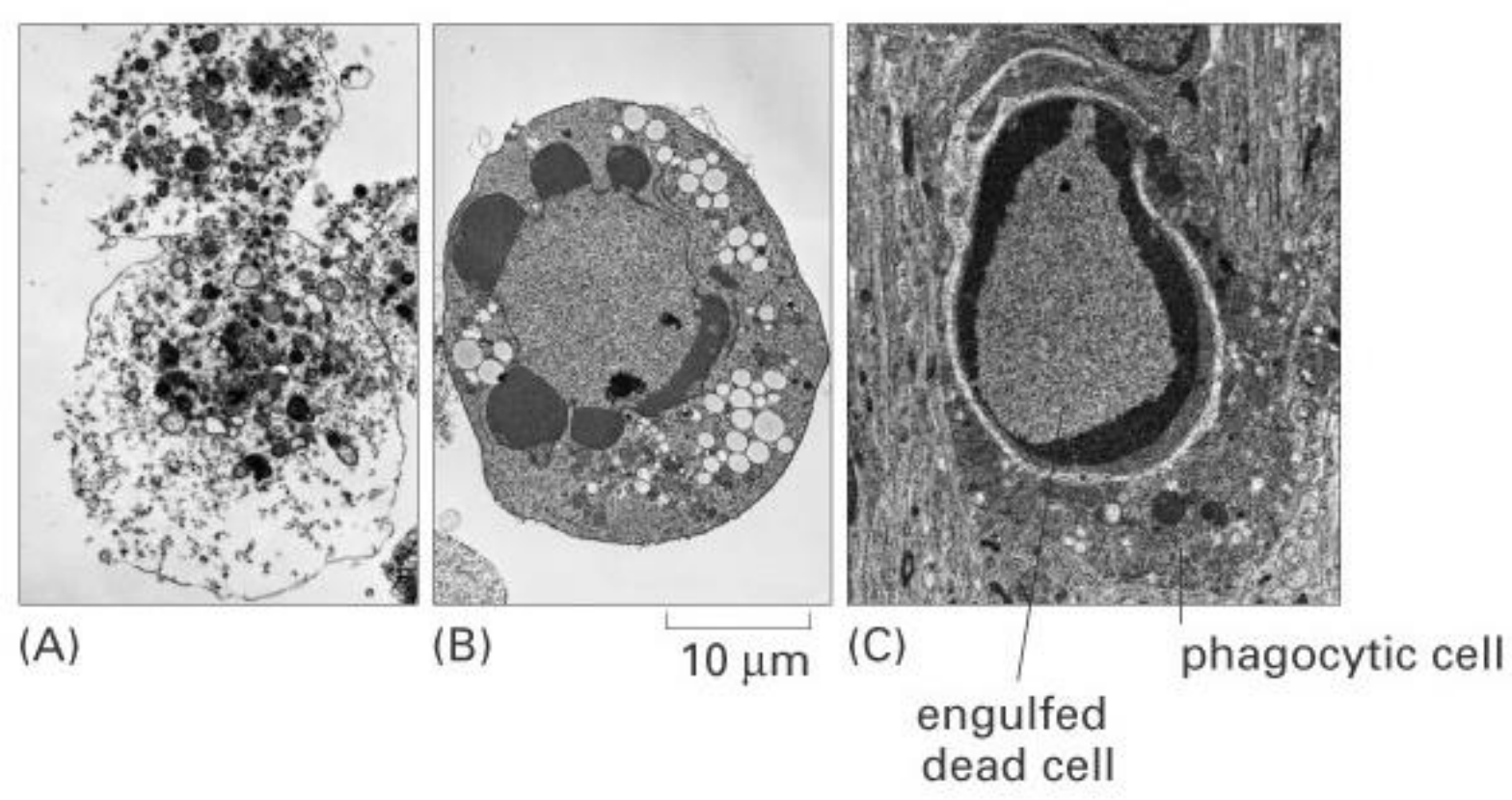
Apoptosis: the necessity for cell death in multicellular organisms

- Embryonic morphogenesis
- Killing by immune effector cells
- Wiring of the developing nervous system
- Regulation of cell viability by hormones and growth factors (most cells die if they fail to receive survival signals from other cells)

Developmentally-regulated apoptosis



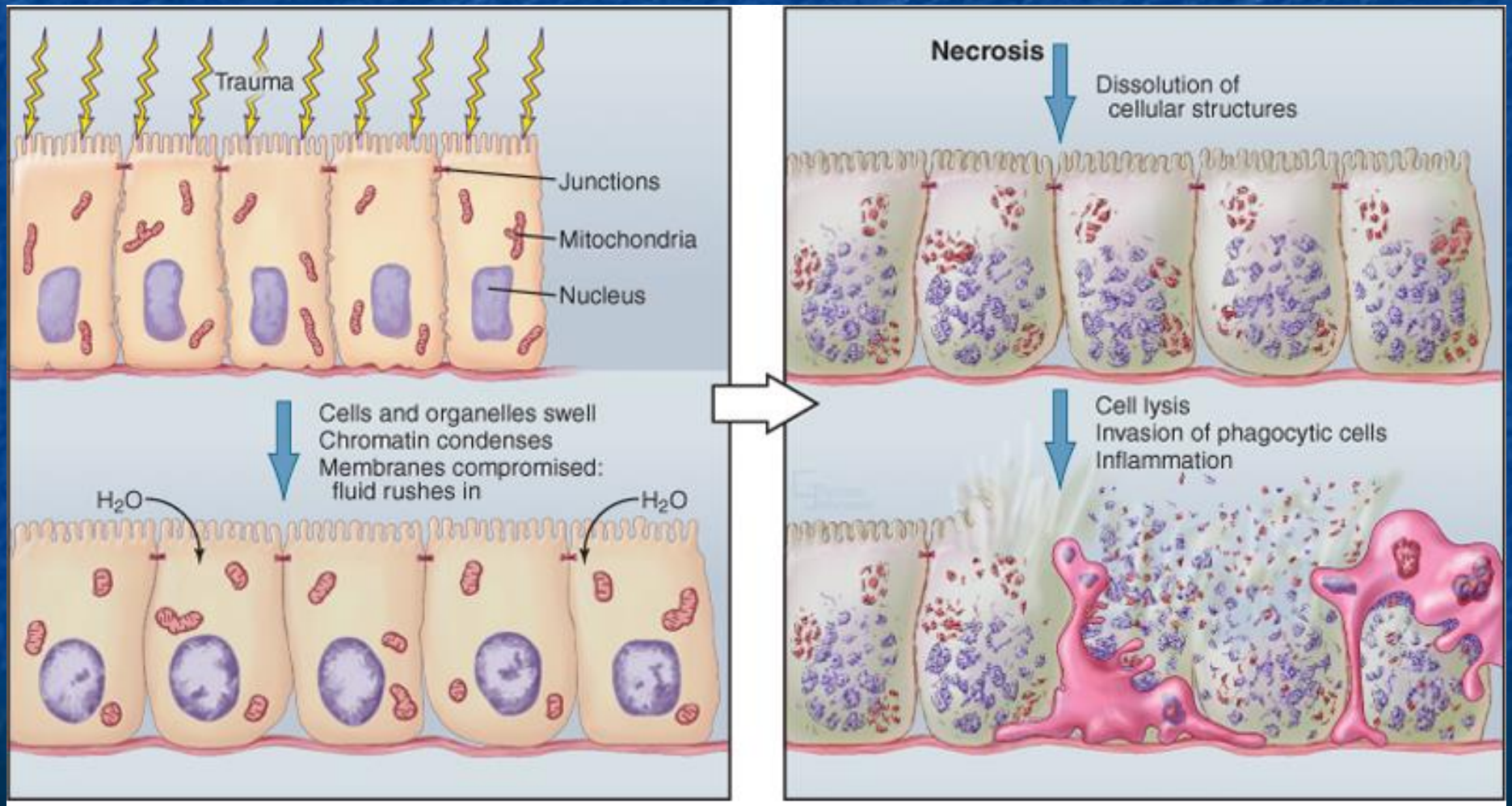
Apoptosis vs. necrosis



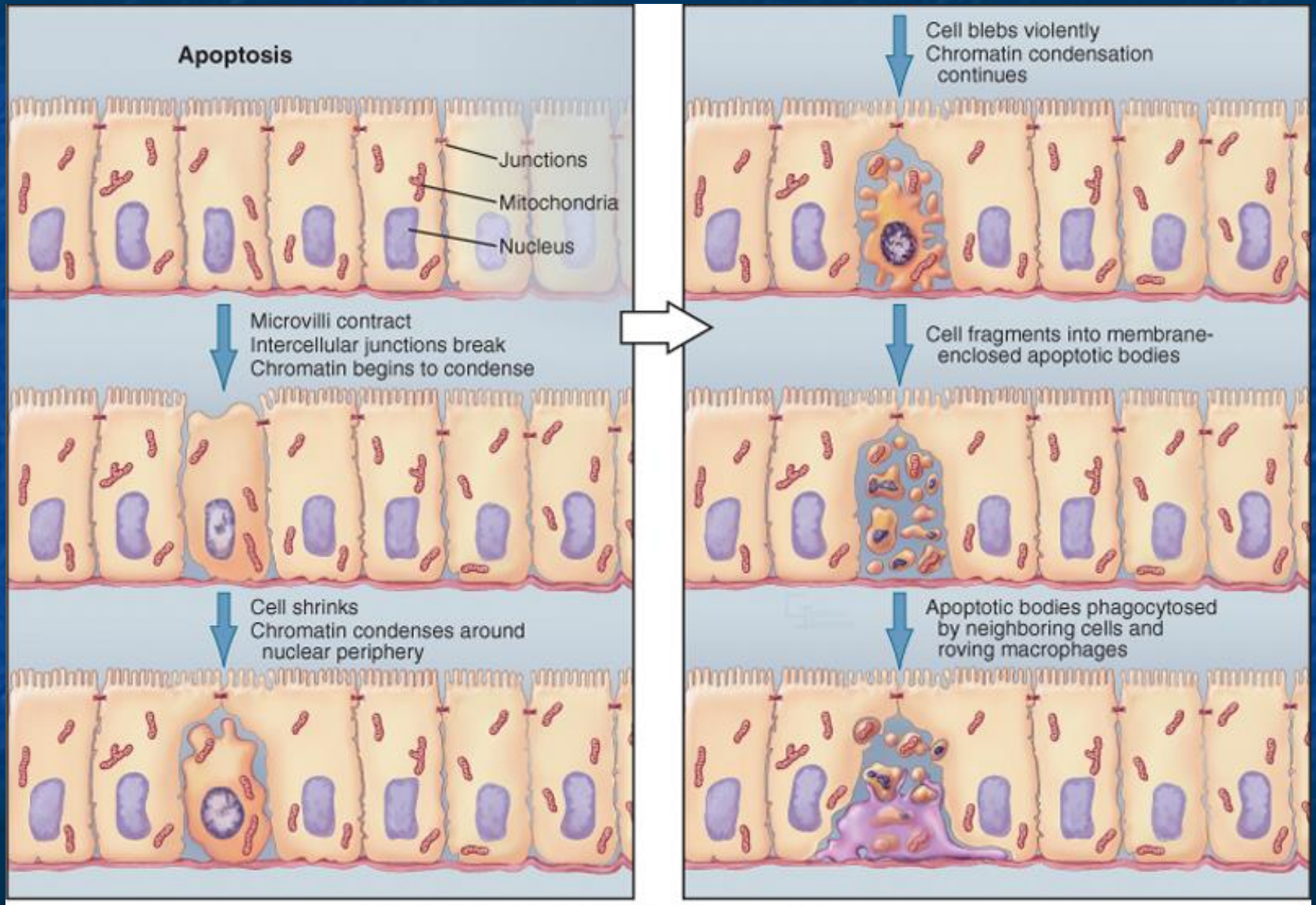
Necrotic cell

Apoptotic cells

Necrosis



Apoptosis



Caspases are specialized proteases that mediate apoptosis

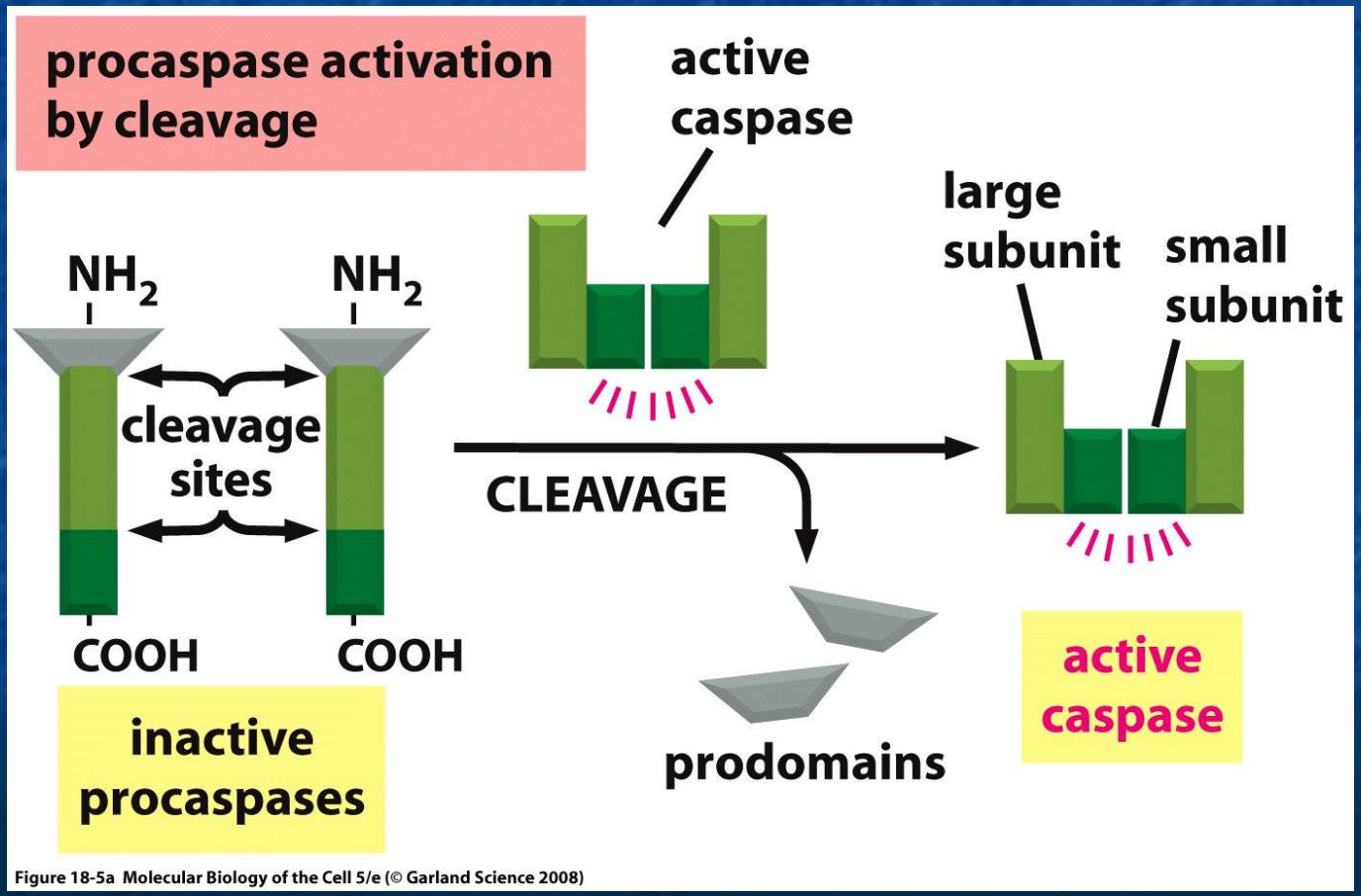


Figure 18-5a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Apoptosis is mediated by an intracellular proteolytic cascade

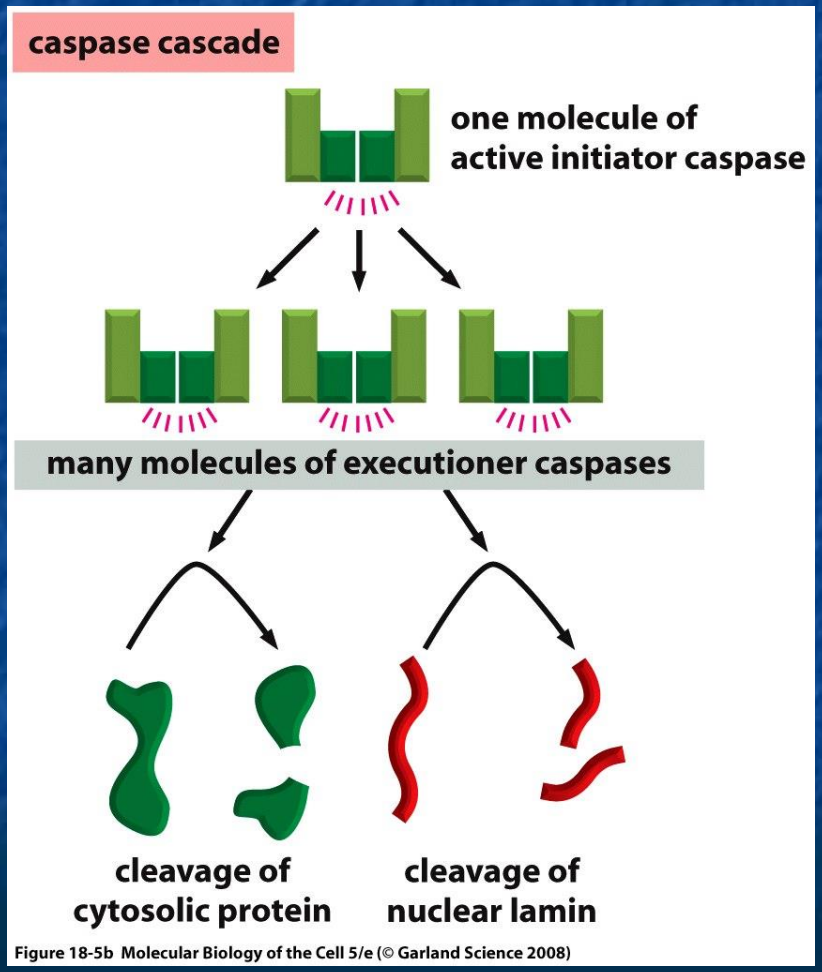


Figure 18-5b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Cell-surface death receptors regulate the extrinsic pathway of apoptosis

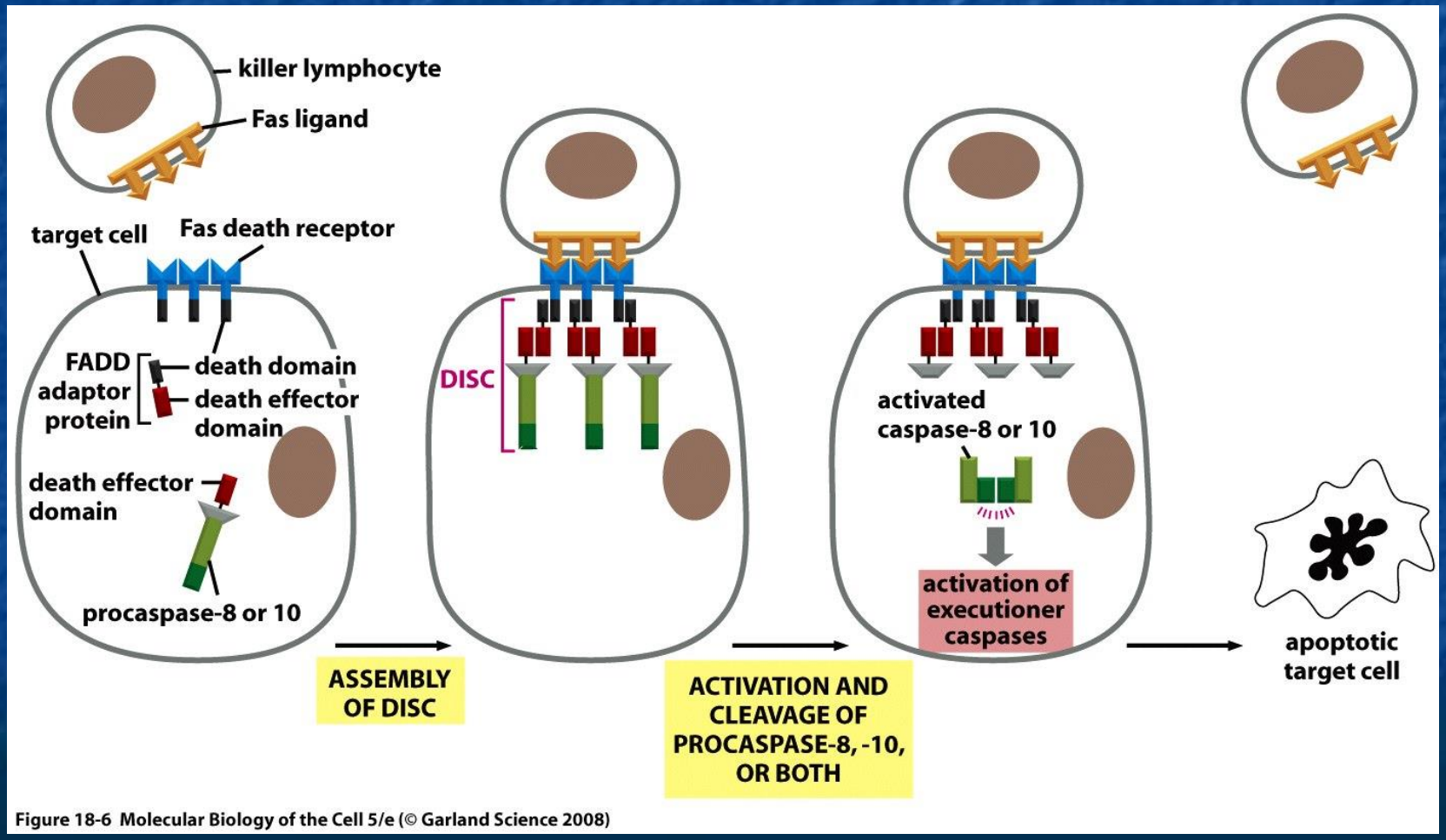


Figure 18-6 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The intrinsic pathway of apoptosis depends on mitochondria

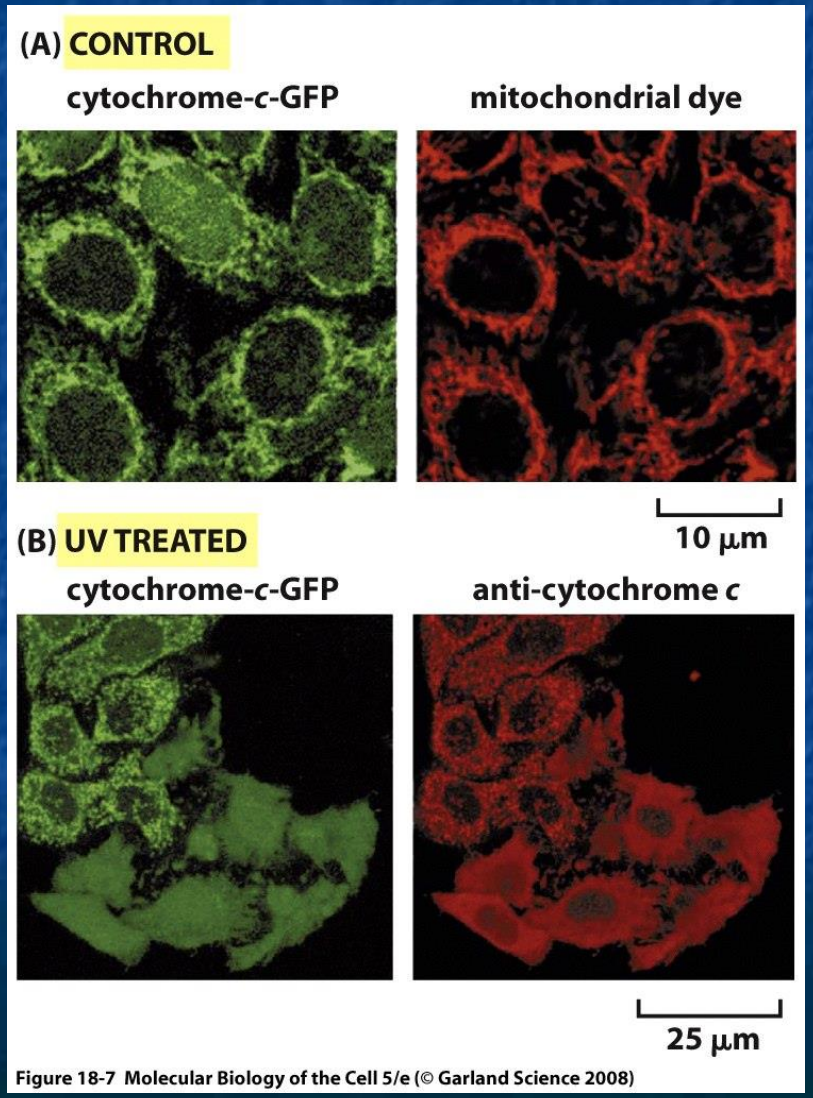


Figure 18-7 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The Bcl-2 family of proteins regulate the intrinsic pathway of apoptosis

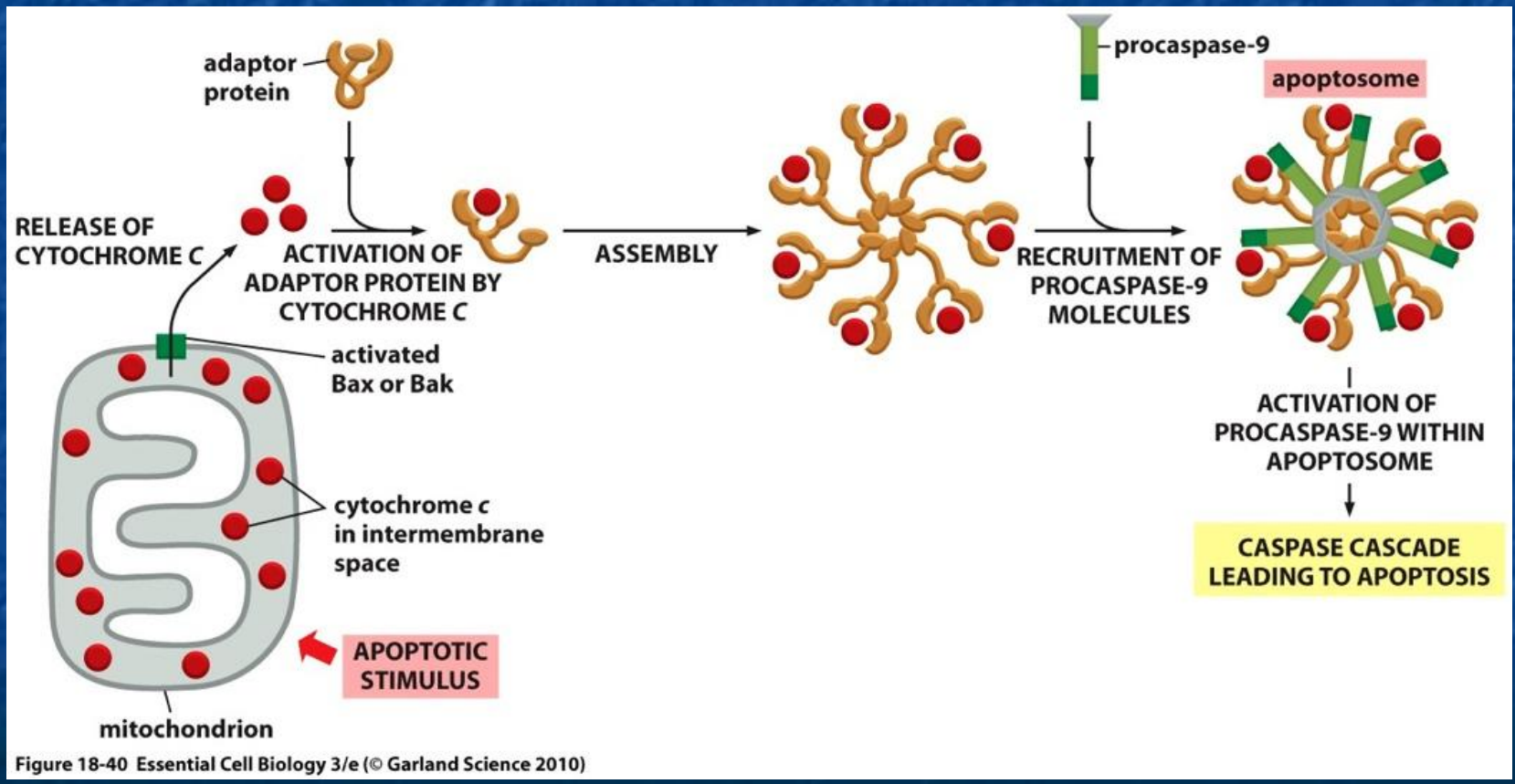
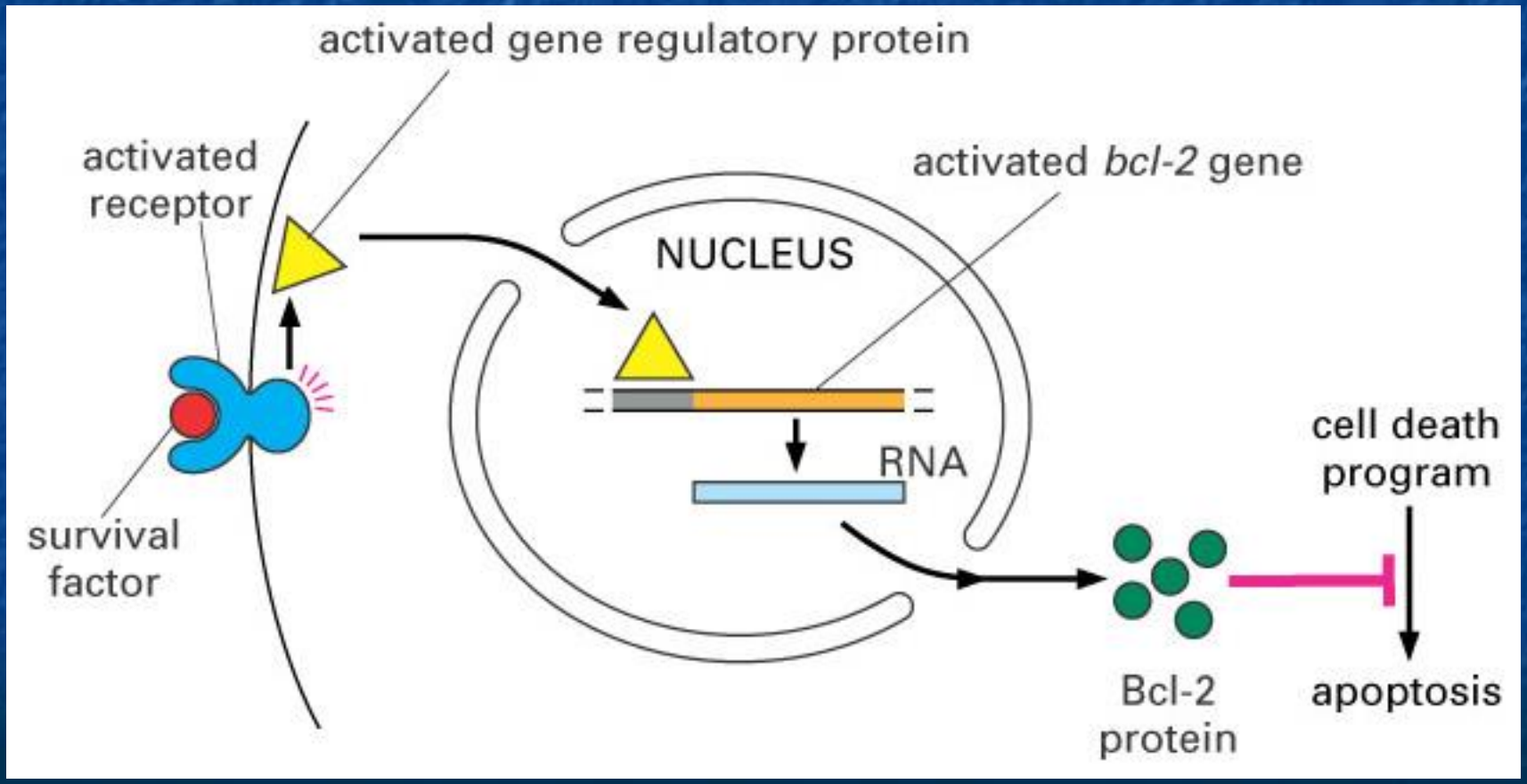


Figure 18-40 Essential Cell Biology 3/e (© Garland Science 2010)

Survival factors suppress apoptosis by regulating Bcl-2 proteins

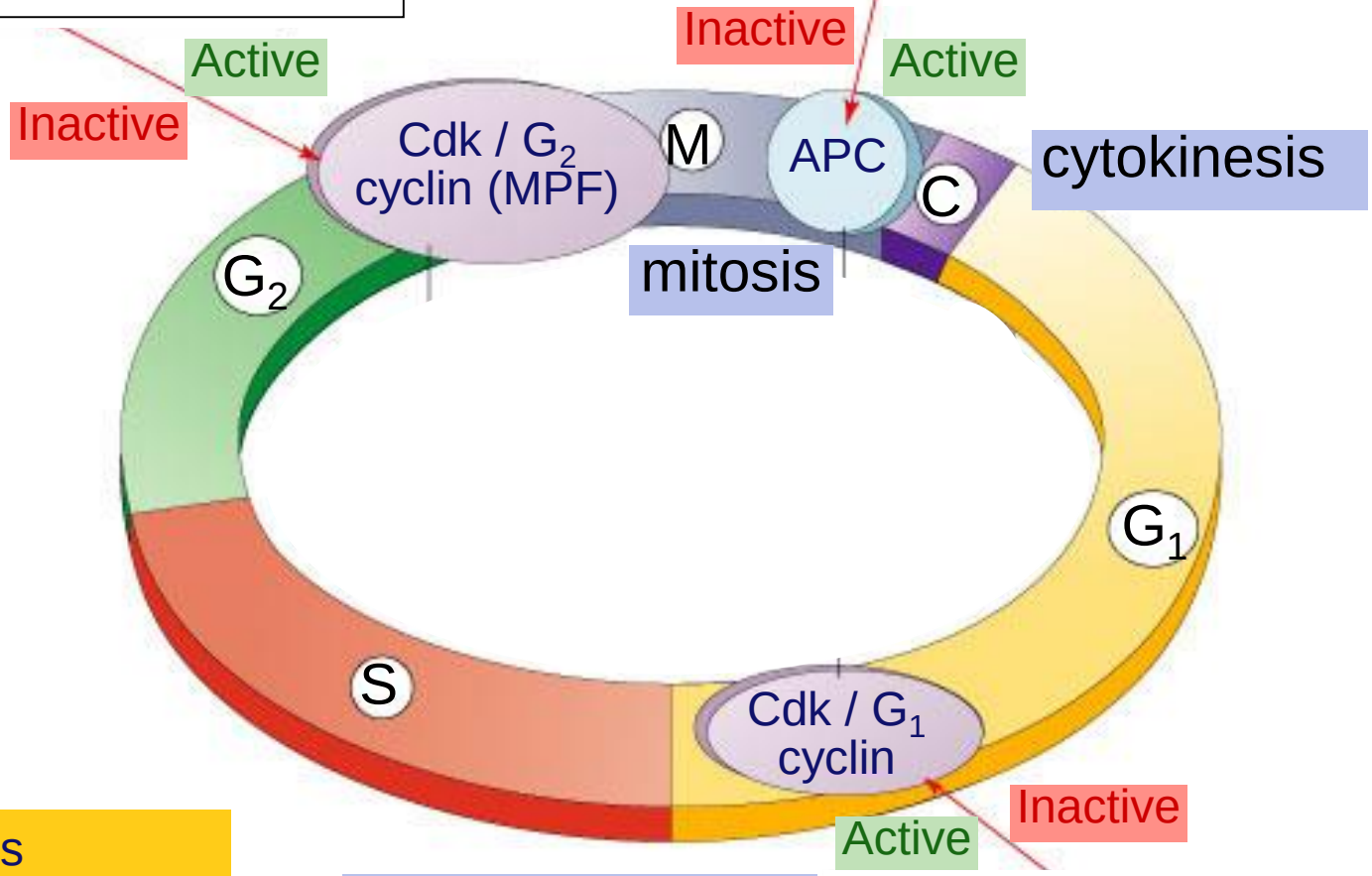


G_2 / M checkpoint

- Replication completed
- DNA integrity

Spindle checkpoint

Chromosomes attached at metaphase plate



G_1 / S checkpoint

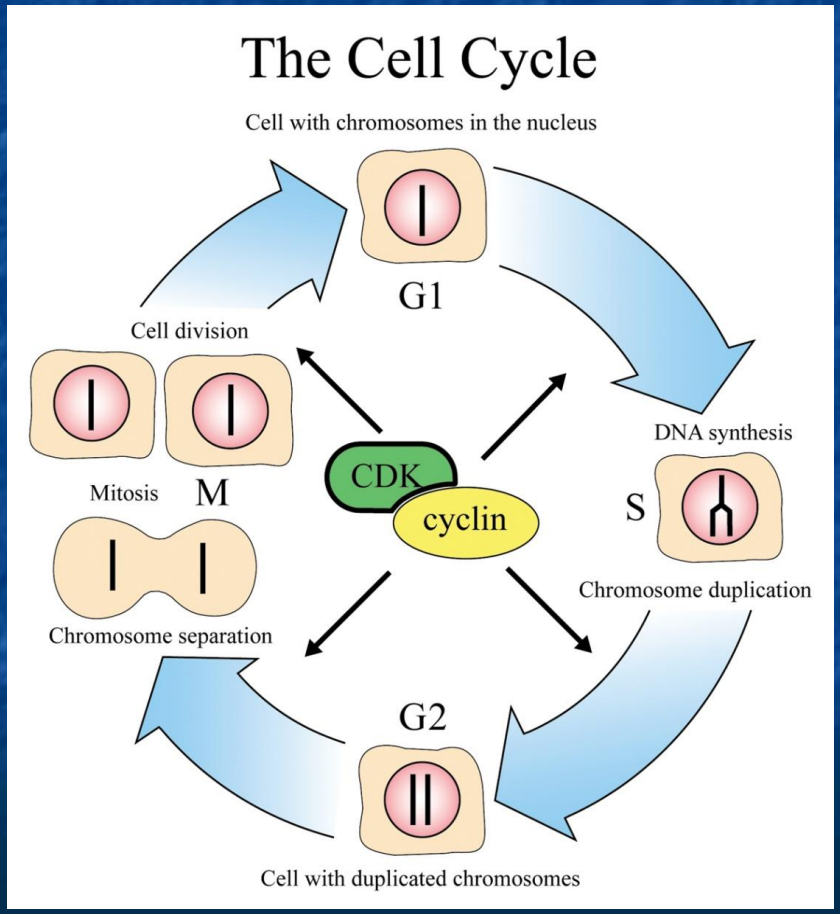
- Growth factors
- Nutritional state of cell
- Size of cell

MPF = Mitosis Promoting Factor

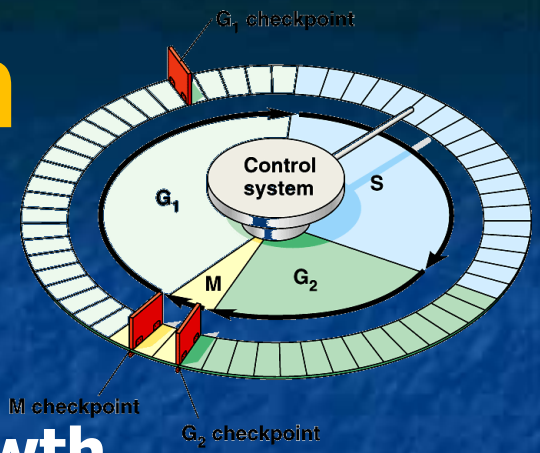
APC = Anaphase Promoting Complex

Cyclin & Cyclin-dependent kinases

- **CDKs & cyclin drive cells from one phase to next in cell cycle**
  - proper regulation of cell cycle is so key to life that the genes for these regulatory proteins have been highly conserved through evolution
  - the genes are basically the same in yeast, insects, plants & animals (including humans)



Cancer & Cell Growth



- Cancer is essentially a failure of cell division control
  - unrestrained, uncontrolled cell growth
- What control is lost?
  - lose checkpoint stops
  - gene p53 plays a key role in G_1/S restriction point
    - p53 protein halts cell division if it detects damaged DNA
      - options:
        - stimulates repair enzymes to fix DNA
        - forces cell into G_0 resting stage
        - keeps cell in G_1 arrest
        - causes apoptosis of damaged cell
    - ALL cancers have to shut down p53 activity

Development of Cancer

- Cancer develops only after a cell experiences ~6 key mutations ("hits")
  - unlimited growth
    - turn on growth promoter genes
  - ignore checkpoints
    - turn off tumor suppressor genes (p53)
  - escape apoptosis
    - turn off suicide genes
  - immortality = unlimited divisions
    - turn on chromosome maintenance genes
  - promotes blood vessel growth
    - turn on blood vessel growth genes
  - overcome anchor & density dependence
    - turn off touch-sensor gene

Tumors

- Mass of abnormal cells
  - Benign tumor
    - abnormal cells remain at original site as a lump
      - p53 has halted cell divisions
    - most do not cause serious problems & can be removed by surgery
  - Malignant tumor
    - cells leave original site
      - lose attachment to nearby cells
      - carried by blood & lymph system to other tissues
      - start more tumors = metastasis
    - impair functions of organs throughout body