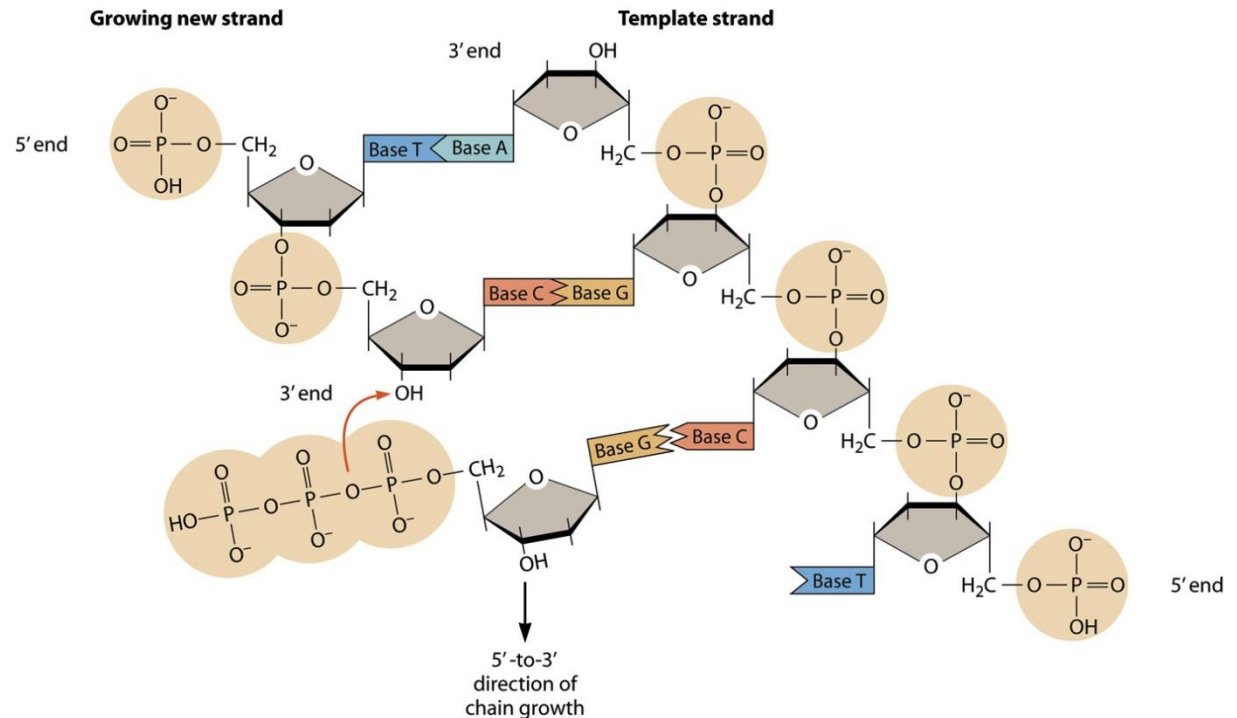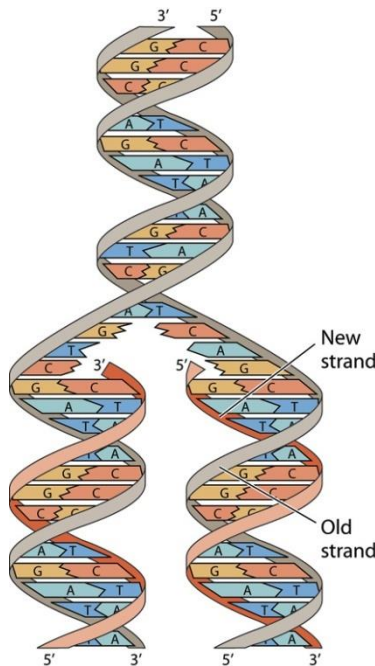Chapter 9

Cells Grow and Reproduce
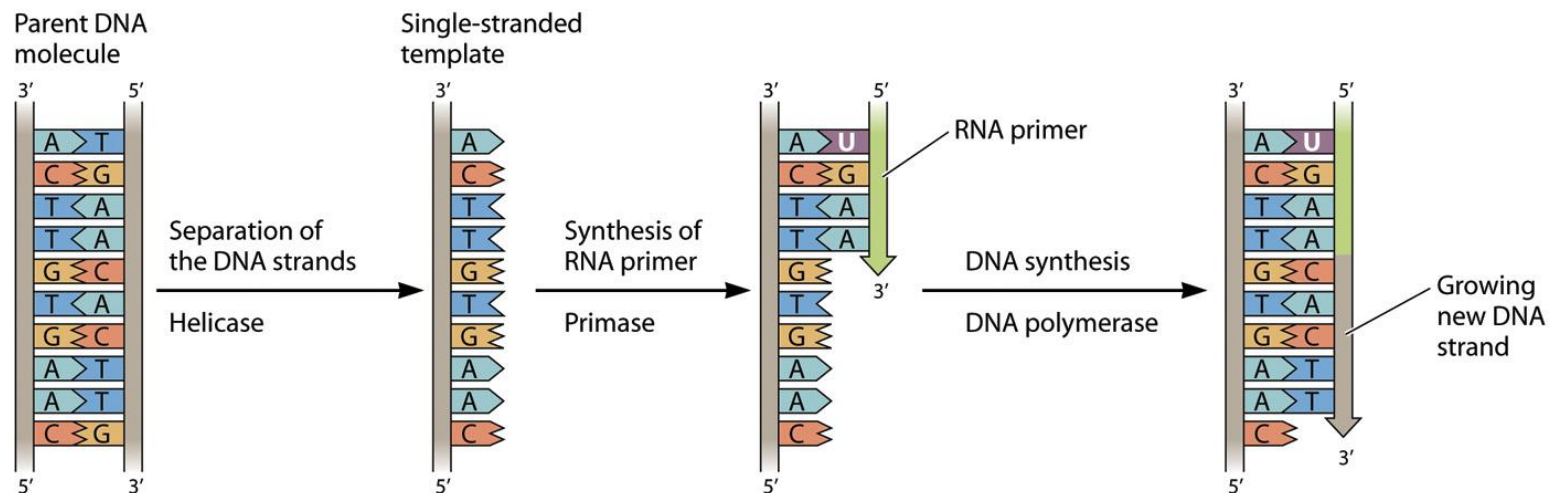
DNA Replication

- DNA polymerase
  - Addition of a nucleotide to the 3' end of a growing strand
  - Use dNTPs as substrate → Release of pyrophosphate
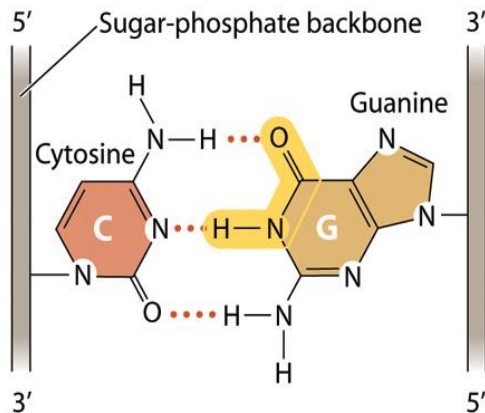
Initiation of Replication

- Replication origin
    - The site where replication starts
    - Binding of several proteins involved in replication
- Helicase
    - Separation of the DNA strands
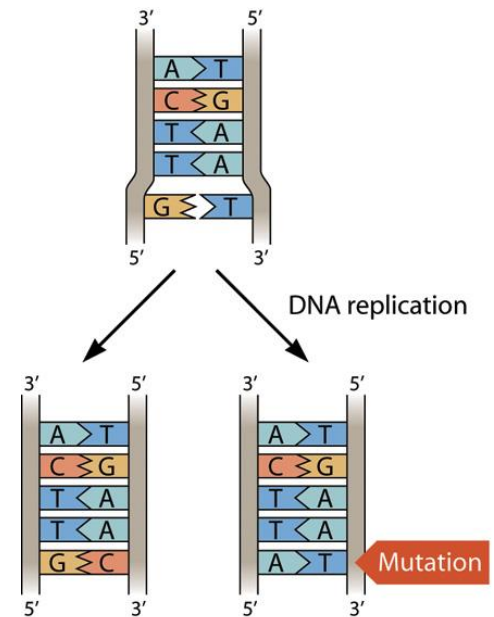- Primase
    - Synthesis of RNA primer

Incorporation of Wrong Nucleotide

- e.g. alternate form of G base pairs with T
- Mismatch → Induction of mutation
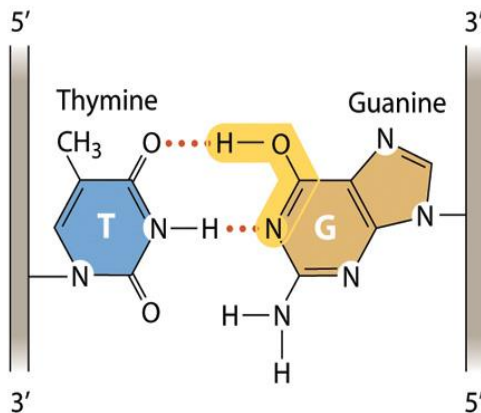
Preventing Mutation

- Proofreading by DNA polymerase

- Several repair systems for mismatches and DNA damage

- Final guard against mutation

  --- "Quality control": no cell division if damaged DNA is present

Rare alternate form of G is incorporated instead of A.

Shift back to normal form of G disrupts base pairing with T.

Polymerase excises mismatched G.

Synthesis proceeds with incorporation of correct base (A).
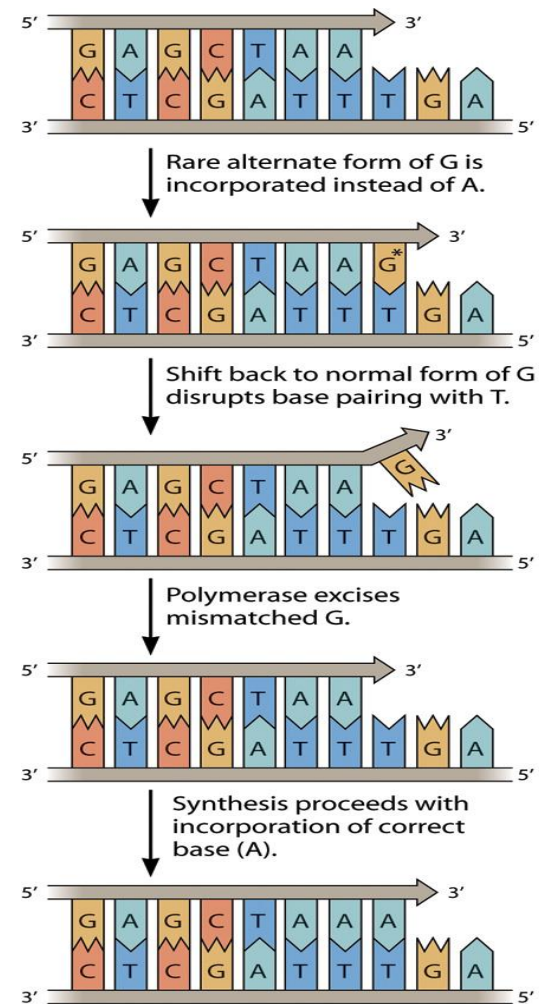
Division of DNA Molecules During Cell Division

- Bacteria
  - Attachment of each DNA to two different spots on the membrane

- Eukaryotes
  - More complex
  - Two copies of 23 different chromosomes (human)

BOX 9.1 *Mitosis, chromosomes, and amniocentesis*

Chromosomes are ordinarily invisible under the microscope because, in their relaxed state, they are such thin structures. When they condense during prophase, however, it becomes possible to see them (under a microscope), and at metaphase they reach the point of greatest condensation. The reason that human chromosomes look like tiny Xs in photographs is that the pictures are taken when chromosomes are in metaphase, so each "chromosome" is really two replicated chromosomes held together at the centromere (see the figure).

When metaphase chromosomes are stained with various stains or fluorescent dyes, patterns of bands become visible. Each chromosome has a unique pattern that can be used to identify it. In fact, it is possible to detect certain kinds of genetic problems by studying stained metaphase chromosomes and looking for irregularities in the banding patterns.

Pregnant women sometimes undergo a diagnostic procedure called **amniocentesis**, in which technicians examine the banding patterns of chromosomes from fetal cells and screen for certain genetic abnormalities. During amniocentesis, intrauterine fluid is drawn, and fetal cells are isolated from the fluid. These cells are normally present in amniotic fluid and are not forcibly removed from the developing fetus.

The cells are put into a test tube and treated with drugs that halt their divi-

sion process in metaphase, and their chromosomes are stained and photographed. The banding patterns are then used to identify the chromosomes, and the chromosomes are inspected for visible problems. This type of examination does not reveal single-base changes or other small changes in the DNA, but it can show major problems, like chromo-

somal rearrangements, missing chromosomes, or extra chromosomes. It also reveals the gender of the baby (see chapter 10). The staining of chromosomes and examination of the resulting banding patterns is called karyotyping, and the pattern obtained from a given individual is that person's **karyotype**.
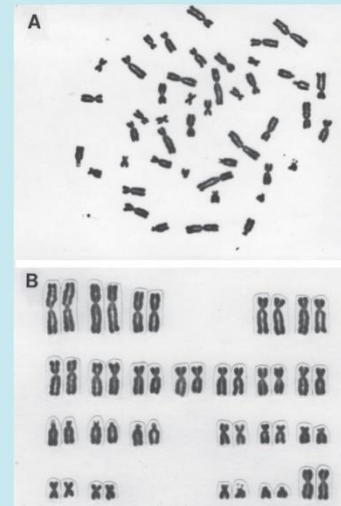
Human metaphase chromosomes. (A) Light micrograph. (B) Paired and sorted. (Photographs copyright Craig Holmes/Biological Photo Service.)

Chromosome

- Tightly packed complex of DNA and histone proteins

Metaphase chromosome — 1400 nm

Condensed scaffold-associated chromatin — 700 nm

Interphase: extended scaffold-associated chromatin

Chromosome scaffold — 300 nm

30-nm chromatin fiber of packed nucleosomes — 30 nm

"Beads-on-a-string" form of chromatin — 11 nm

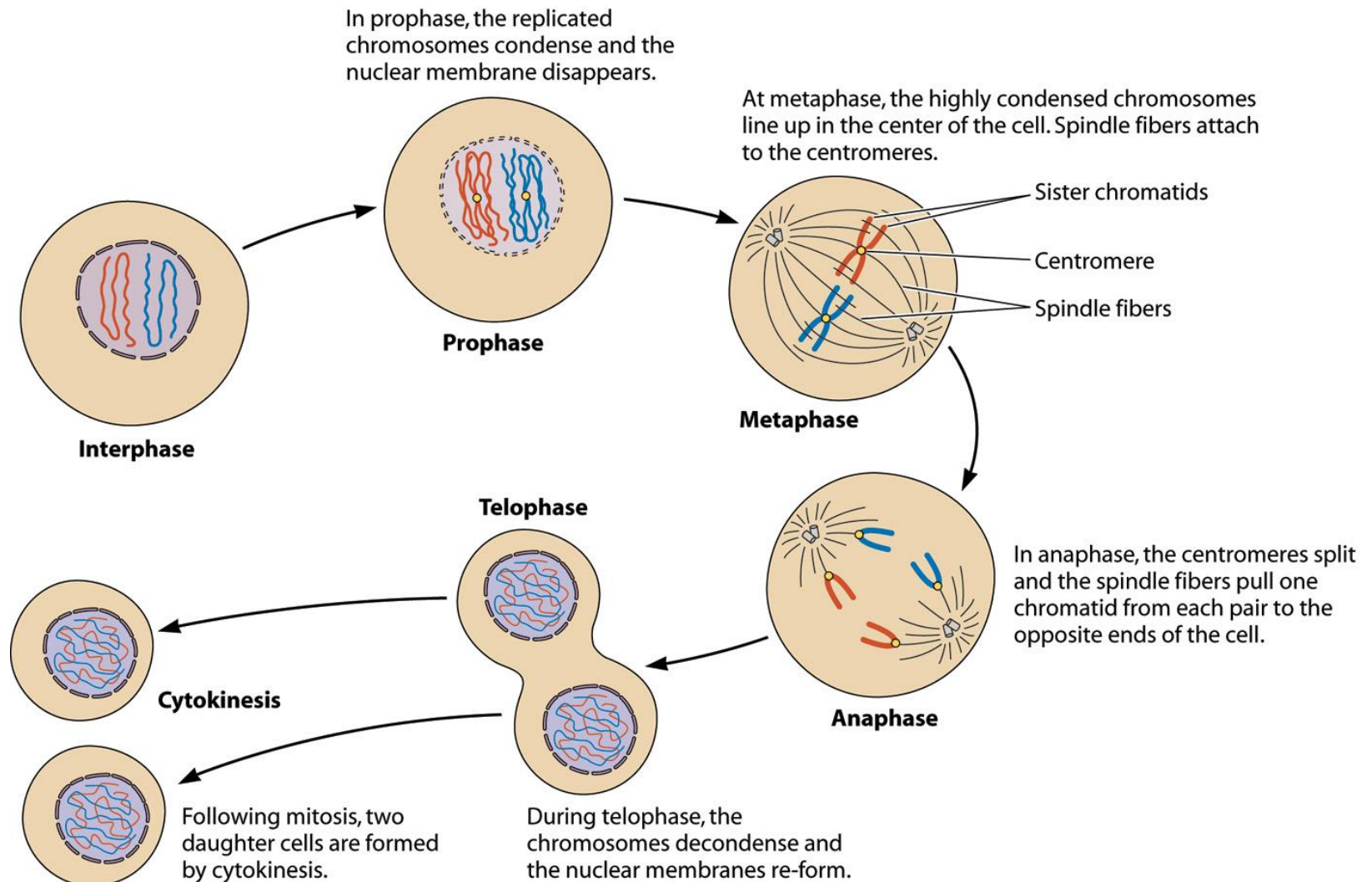Short region of DNA double helix — 2 nm
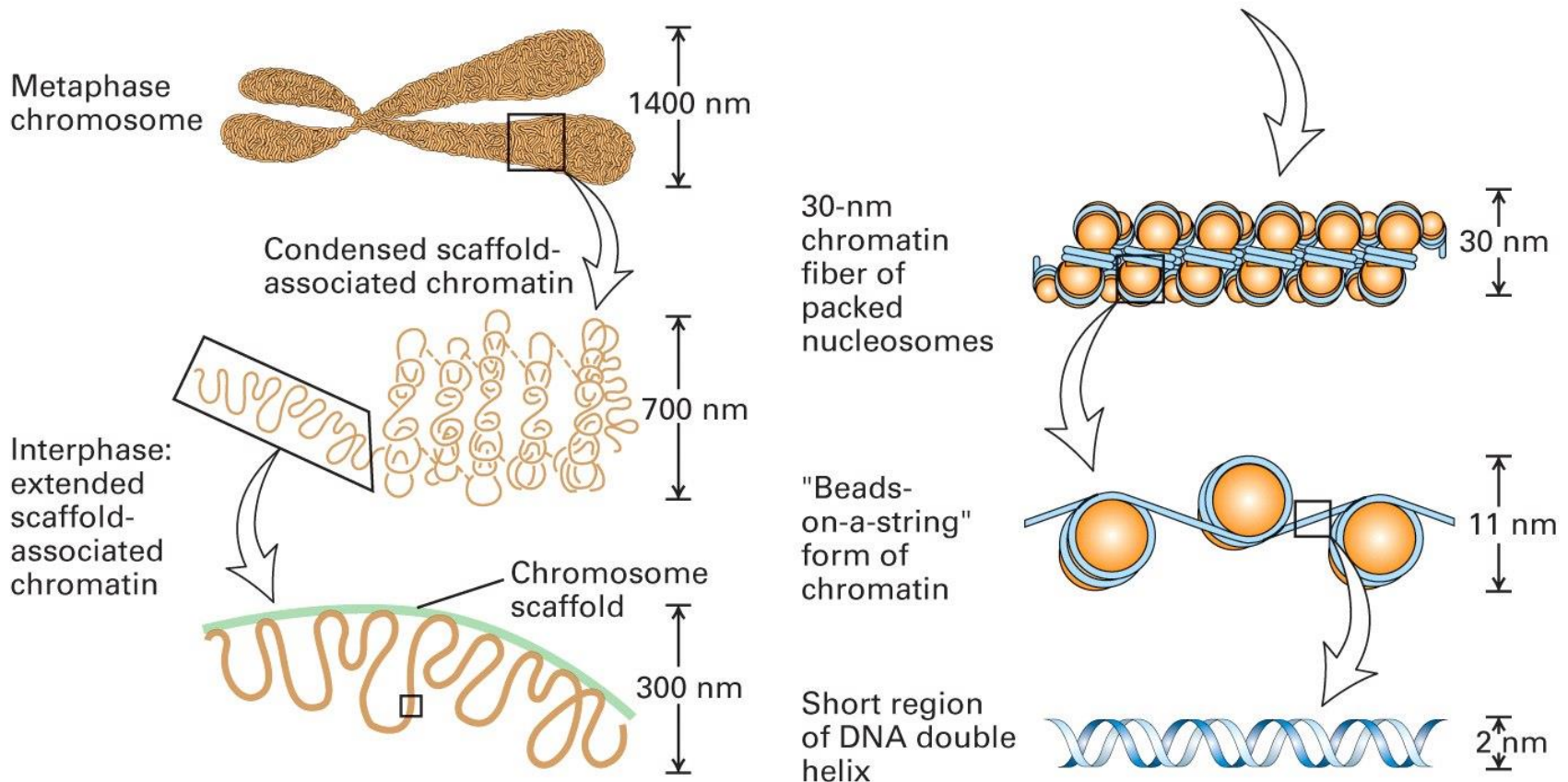
Mitosis

- Distribution of chromosome to daughter cells
- Mitosis does not begin until DNA replication has already occurred.
- Setting the stage after DNA replication
  - Connected two daughter chromosome after DNA replication
    - Joined at centromere (unique DNA sequence) via a protein clamp
    - Kinetochore:
      - centromere + centromere proteins
    - Chromatid
      - --- each identical DNA molecule

Nucleus

DNA replication

Cell with one pair of chromosomes. The chromosomes are relaxed and spread out in the nucleus.

Centromere

Cell is ready for mitosis.

After DNA replication, the two daughter DNA molecules stay clamped together at their centromeres. The replicated chromosomes are still relaxed and spread out.

Chromatid

Spindle fibers
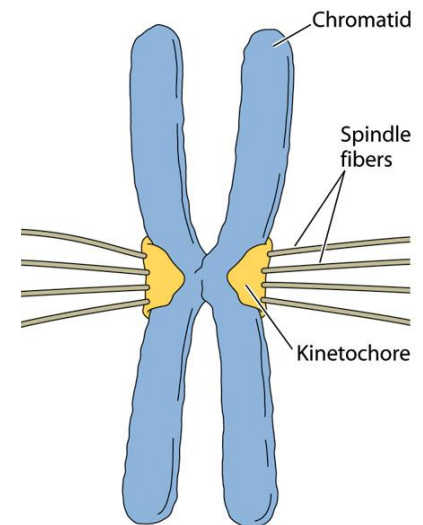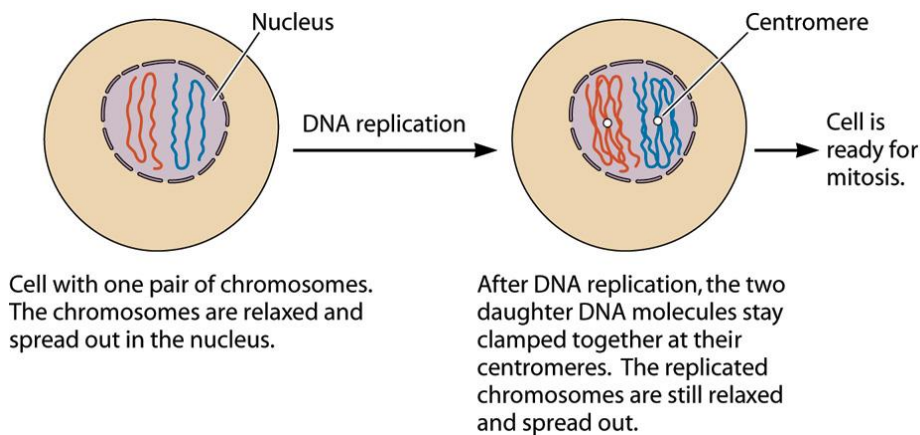
Kinetochore

Mitosis

- **DNA replication**
- **Mitosis**
  - Prophase
    - Condensation of chromosomes and disappearance of nuclear membrane
  - Metaphase
    - Alignment of chromosome in the center
    - Pulling by spindle fibers attached to the kinetochore
  - Anaphase
    - Splitting of chromatids and pulling to the opposite ends of the cell
  - Telophase
    - Decondensation of chromosome
    - Formation of new nuclear membrane
- **Cytokinesis**
  - Cell division after mitosis
- **Interphase**
  - The time between cell division and the next mitosis (G_1, S, G_2)

Mitosis and Cytokinesis

In prophase, the replicated chromosomes condense and the nuclear membrane disappears.

Prophase

At metaphase, the highly condensed chromosomes line up in the center of the cell. Spindle fibers attach to the centromeres.

Sister chromatids

Centromere

Spindle fibers

Metaphase

Interphase

Telophase

In anaphase, the centromeres split and the spindle fibers pull one chromatid from each pair to the opposite ends of the cell.

Anaphase

Cytokinesis

Following mitosis, two daughter cells are formed by cytokinesis.

During telophase, the chromosomes decondense and the nuclear membranes re-form.

The Cell Cycle

- Cell Cycle
  - S phase:
    - --- DNA synthesis
      (DNA replication)
  - M phase:
    - --- mitosis
  - G_1, G_2 :
    - --- G stands for gap
      between S and M phase
   - --- Cell growth
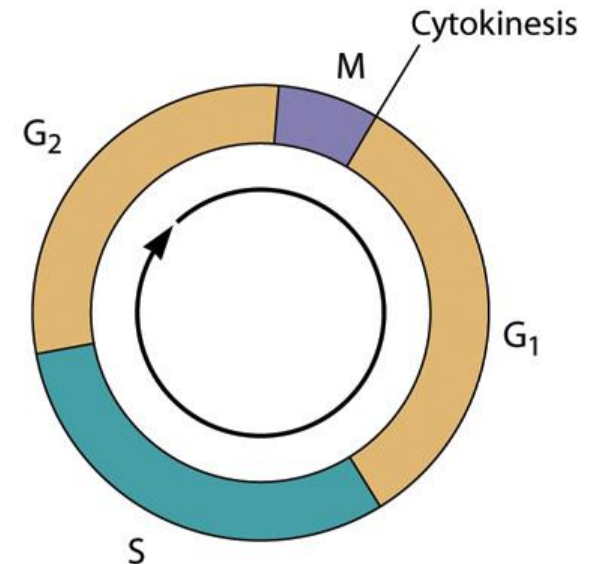
The Cell Cycle

- Regulation of cell cycle
  - Restriction point : late G1
    - With growth factor → S phase
    - Without growth factor → G0 : metabolism without growing

      e.g. platelet-derived growth factor during blood clotting
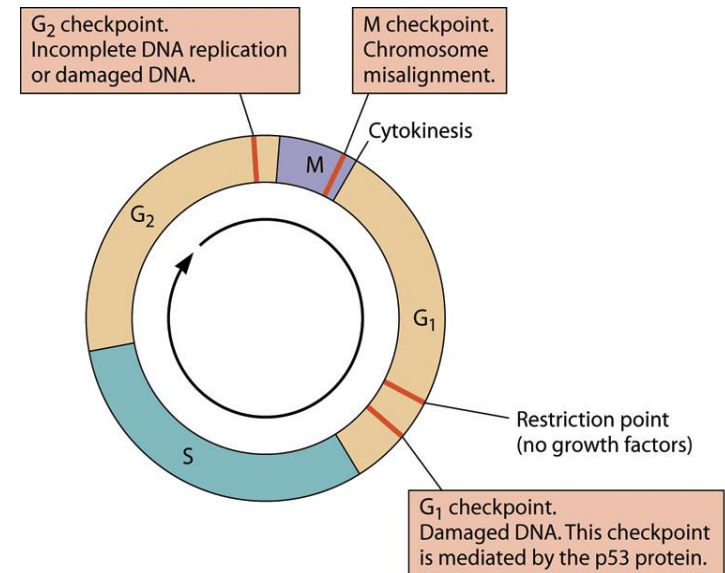
      → Growth of skin fibroblasts
  - Ras protein
    - Activated by many growth factors
      - Many growth factor receptors transmit their signals through Ras.
    - Signal transduction to induce DNA synthesis

Cytokinesis

M

G2

G0

S

Restriction point (no growth factors)
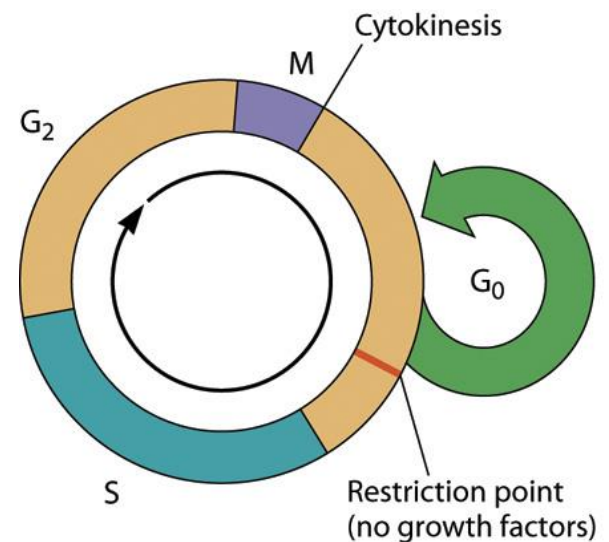
Cell Cycle Checkpoints

- Roles of cell cycle checkpoints
  - Prevent entry into the next phase before the completion of the previous phase
  - DNA damage checkpoints
- Cell cycle checkpoints
  - G1 check point
    - *p53* : activated by damaged DNA
      - → activates the G1 check point
      - → stops DNA replication
    - Success in damage repair
      - → proceeds DNA replication
    - Fail in damage repair
      - → The p53 induces apoptosis. (programmed cell death)
  - G2 check point
    - Activated by damaged DNA and unreplicated DNA
  - M check point
    - Incorrect mitosis

G_2 checkpoint. Incomplete DNA replication or damaged DNA.

M checkpoint. Chromosome misalignment.

Cytokinesis

G_2

M

G_1

S

Restriction point (no growth factors)

G_1 checkpoint. Damaged DNA. This checkpoint is mediated by the p53 protein.

Unregulated Cell Division : Cancer

- Cancer: caused by failure in regulation of cell division
  - Carcinoma
    - Originating from epithelial cells (85% of all cancers)
  - Sarcoma
    - Originating from cells of connective tissue, bone, or muscle tissue
  - Leukemia
    - Originating from white blood cells (leukocytes)
  - Adenocarcinoma
    - Originate from glandular tissue
  - Glioma and astrocytoma
    - Cancers of the nonneuronal cells of the brain
- Tumor: a mass of cancer cells derived from a single parent cell
  - Benign: no invasion
  - Malignant: invasion of surrounding tissue
    - Metastasis: migrate to new sites and establish new tumors

The accumulation of mutations in a single cell can lead to cancer

- Oncogenes
  - Mutant genes that promote cell division
    - Genes in signaling pathway to cell division
      (e.g. *ras*, platelet-derived growth factor receptor gene)
  - Mutation in *ras*
    - constitutively active → cell division
    - 20% of human cancer
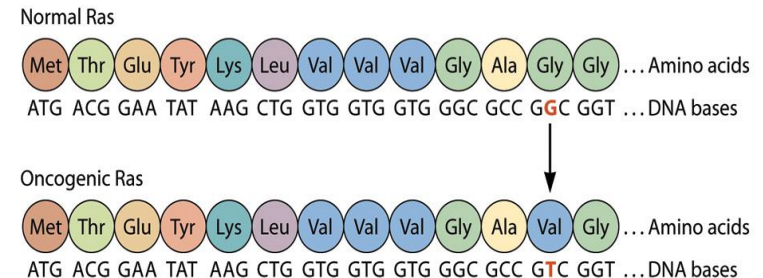
- Tumor suppressor genes
  - Genes that halt cell replication
    - Mutation causes cancer.
  - *p53* : DNA damage check point protein
    - 50% of human cancers including leukemia, brain tumor, breast, colon, and lung cancer
  - *BRCA1* and *BRCA2* genes
    - Breast cancer
 - *MADR2* and *APC* genes
    - Colon cancer

Genes Involved in Cancer

Table 9.1 Some cancer genes and the normal physiological roles of their products

Gene	Normal physiological role
Oncogenes	
sis	Growth factor
erbB, fms, neu	Growth factor receptors
ras, src, abl	Signal transmission within the cell
bcl2	Blocks programmed cell death
myc, fos, myb	Regulators of transcription
Tumor suppressor genes	
rb	Regulation of replication and transcription
p53	Regulation of cell division cycle; stops cells from dividing if their DNA is damaged, allowing time for repair; initiates programmed cell death if DNA is not repaired

Development of Cancer

- Accumulation of mutations during cancer development
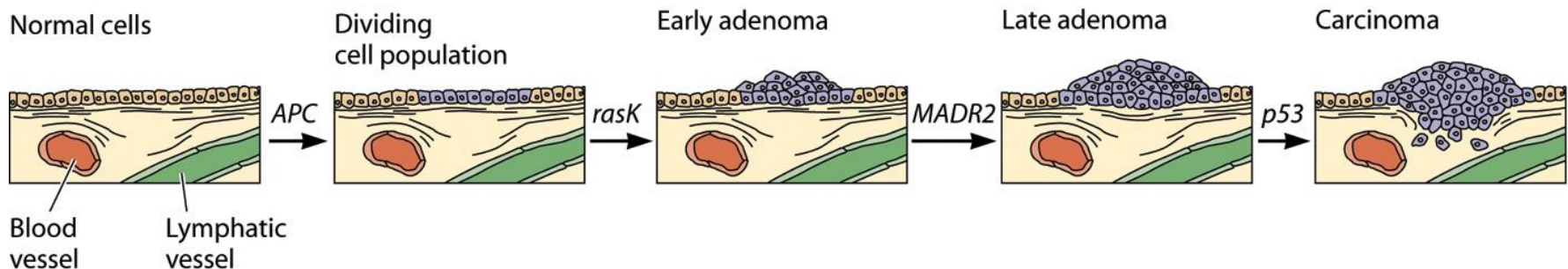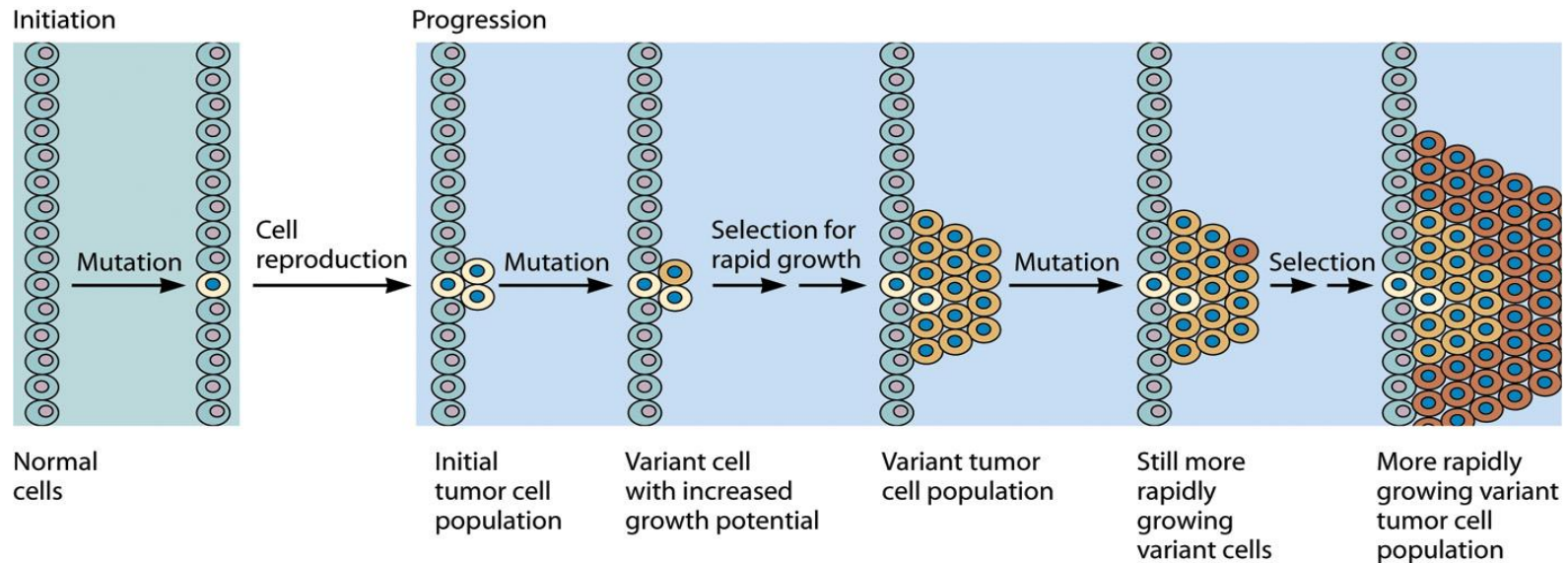
# Inherited Mutation in Tumor Suppressor Genes

- ## Mutation and inheritance
  - Mutations in somatic (soma, body) cells
    - No inheritance
  - Mutations in reproductive cells (eggs, sperm)
    - Inheritance

- ## Inherited mutations and cancer
  - Breast tumor suppressor genes
    - *BRCA1*: involved in DNA repair
    - Mutant *BRCA1* gene
      - 80% chance of developing breast cancer (normal gene: 10%)
      - 40% chance of developing ovarian cancer
    - Inherited mutations of *BRCA1* and *BRCA2* are involved in only 5 to 10% of breast cancers → sporadic mutations are the major cause

Breast Cancer
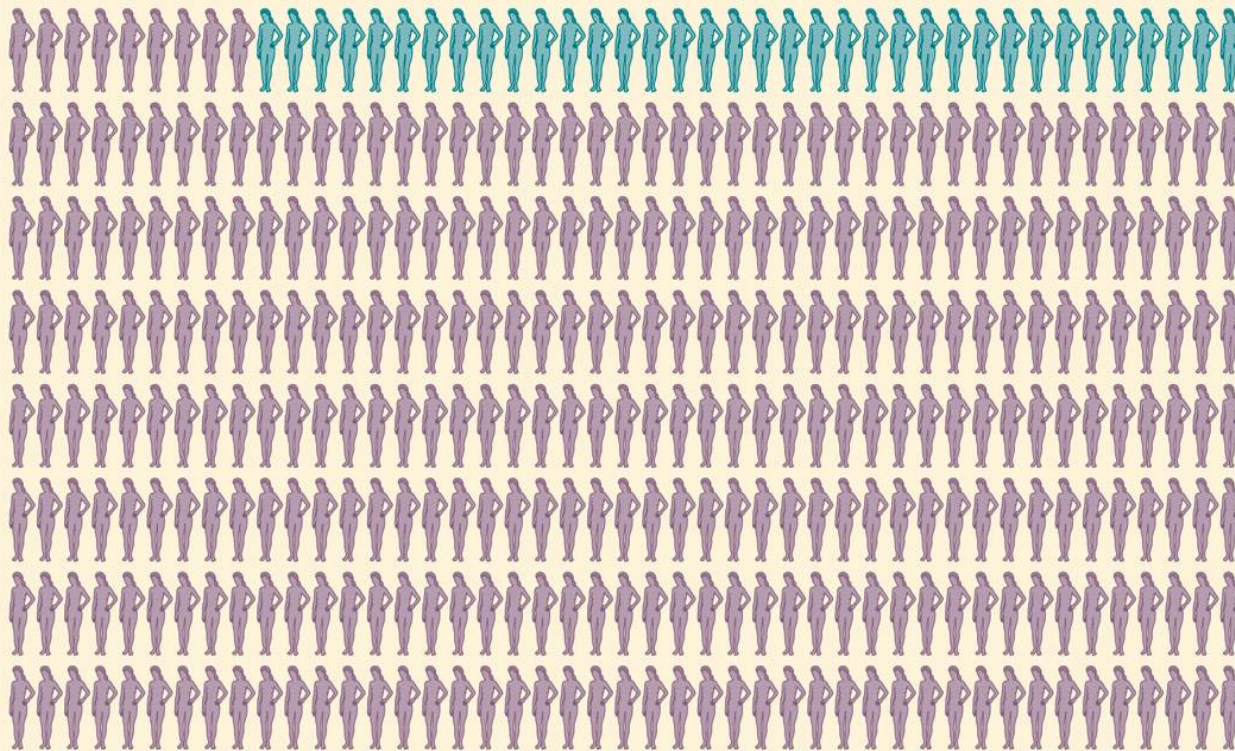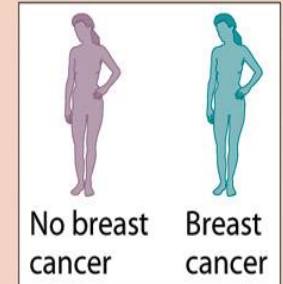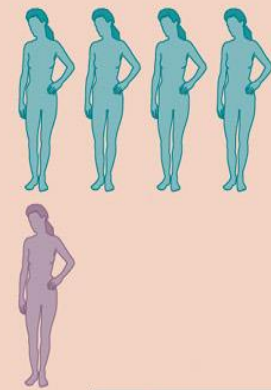
DNA Damage and Repair

- ## DNA damaging agents
  - Mutagens : mutation-promoting agents
  - Carcinogen : cancer-inducing agents
- ## Environmental carcinogens
  - UV
    - Thymine dimer formation
      → blocking transcription
      and DNA replication
  - DNA-binding chemicals
    - Benzopyrene
      - Smoke from cigarette, burning leaves, diesel exhaust etc.
      - Bind to DNA G residue and induce mutation
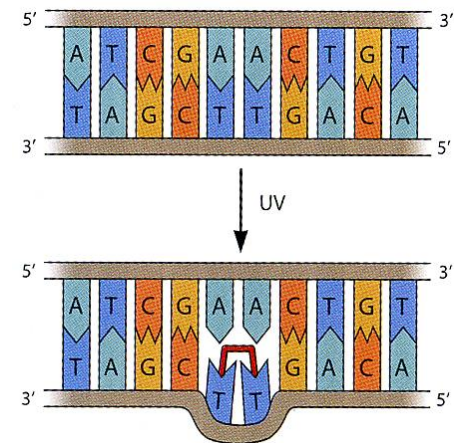
Repair System

- **Mismatch repair**
  - as described before
- **Excision repair**
  - Repair distorted DNA (T-T, benzopyrene binding)
  - Excision of damaged region by nuclease and helicase, and repair by DNA polymerase
  - Xeroderma pigmentosum (XP)
    - Disease
      - Mutation in excision repair system
      - Can not repair T-T
    - Extreme sensitive to UV → skin cancer

Damage recognition
Nuclease cleavage

Helicase

Excised
oligonucleotide

DNA polymerase
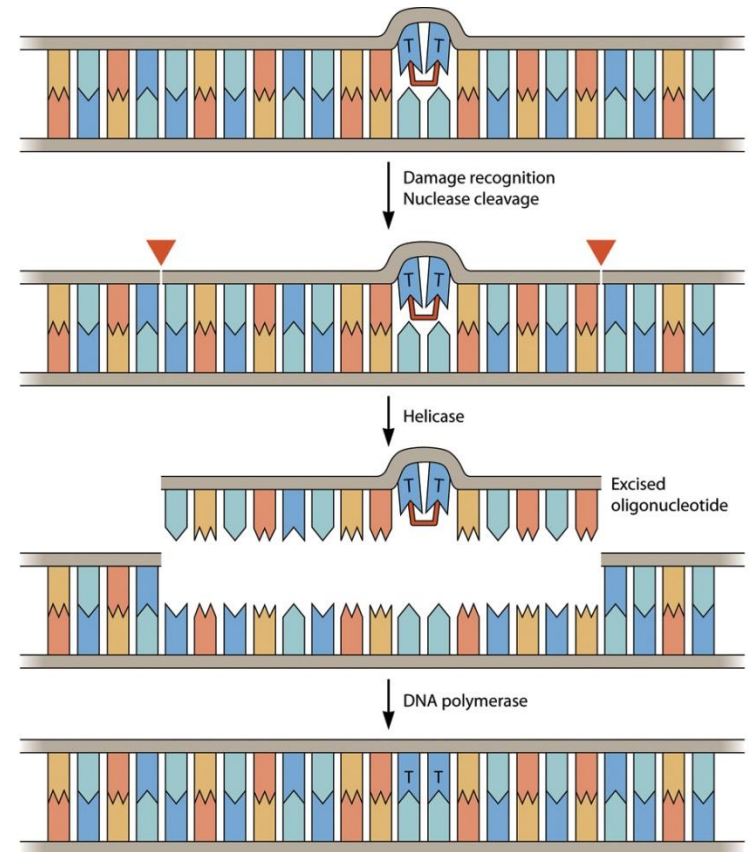
Cancer Drugs

- **Classic anticancer treatment**
  - Targeting rapidly dividing cells
  - Side effects to other fast growing cells
    - Blood cell progenitors, cells lining the digestive tract, hair follicle cells
- **Cancer-specific drugs**
  - Tamoxifen
    - Mimic estrogen : binding to estrogen receptor of estrogen-sensitive cancer cells
  - Herceptin
    - Binding to and inactivate Her2 (receptor for EGF): inhibit the growth of Her2-overproducing breast cancer cells
  - Greevec
    - Inhibition of Abl in chronic myelogenous leukemia