Johns Hopkins Engineering

Molecular Biology

DNA Replication

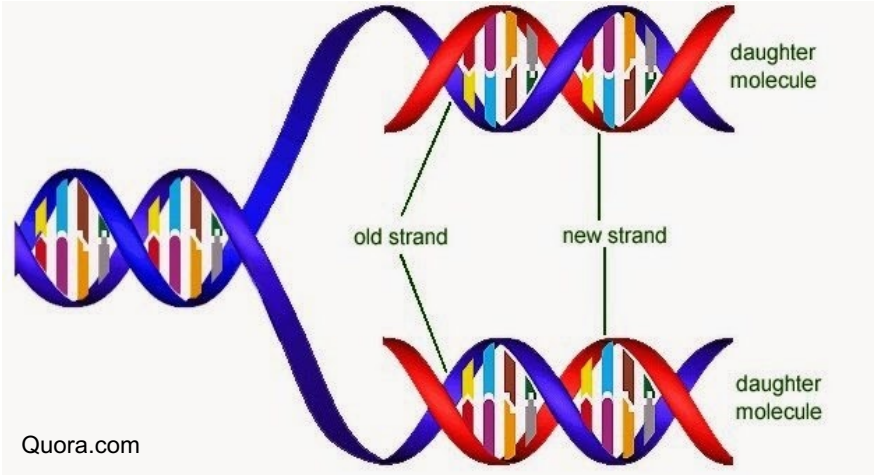
JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING

Outline

- DNA Replication
- DNA Damage and Repair
- Cell Checkpoints
- Cell Death

DNA Replication

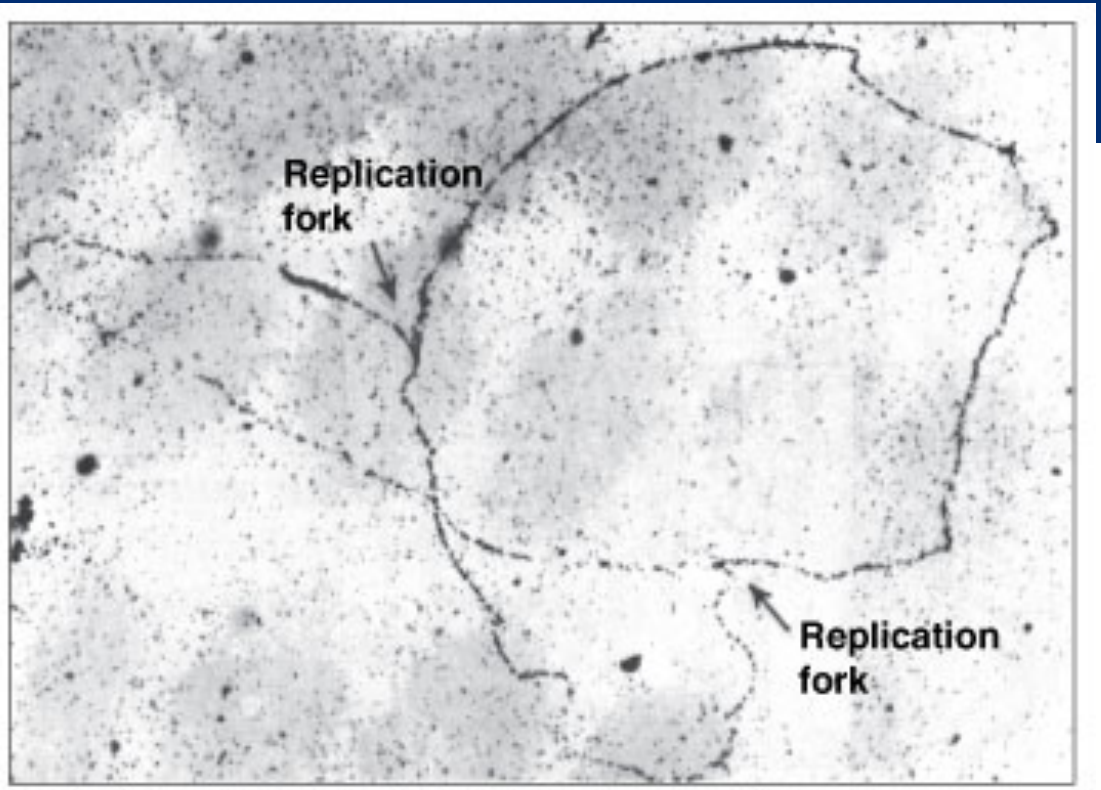
- A central event in the cell cycle
- Mechanism depends on the double-helical structure of DNA
- One strand of every new DNA molecule is derived from the parent molecule and the other is new: this is called **semiconservative replication**
- The two parental DNA strands unwind and each specifies a new daughter strand by base-pairing rules



DNA Replication Is Usually Bidirectional

- DNA replication is especially well understood in *Escherichia coli*
- Replication is very similar in prokaryotes and eukaryotes
- Early biologists studied replication in *E. coli*; They grew cells in a medium containing ^{3}H-thymidine
- They visualized the circular chromosomes by autoradiography and observed **replication forks**
- These are formed where replication begins and then proceeds in *bidirectional fashion* away from the origin

Figure 19-4A



(a) Autoradiograph of *E. coli* DNA replication

Figure 19-4B

Bacterial replication

Single origin of replication

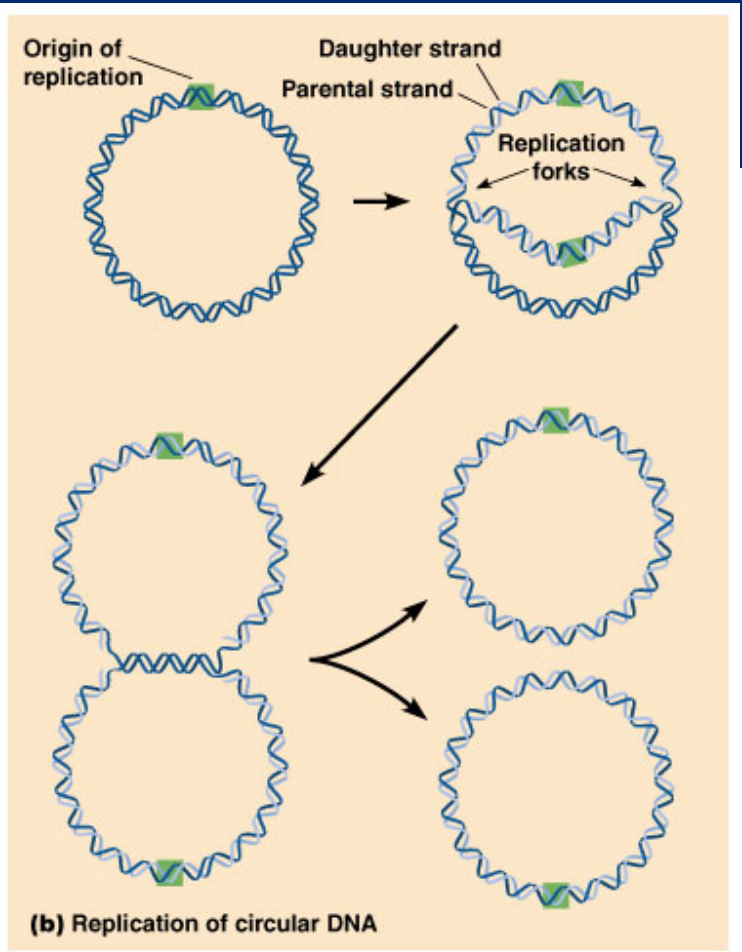
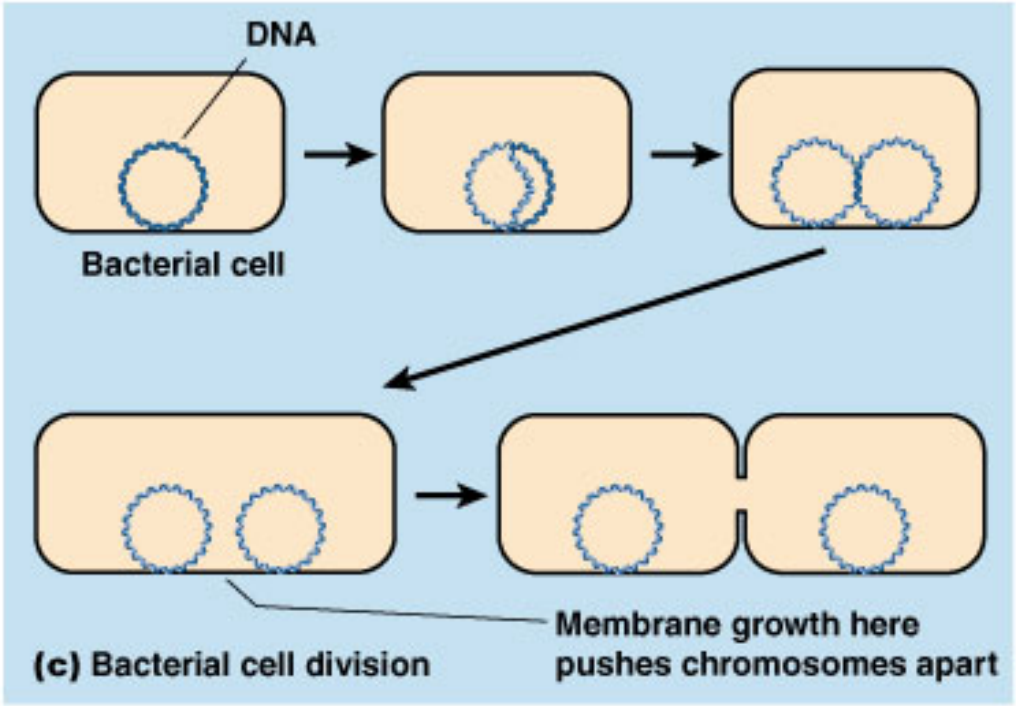


Figure 19-4C



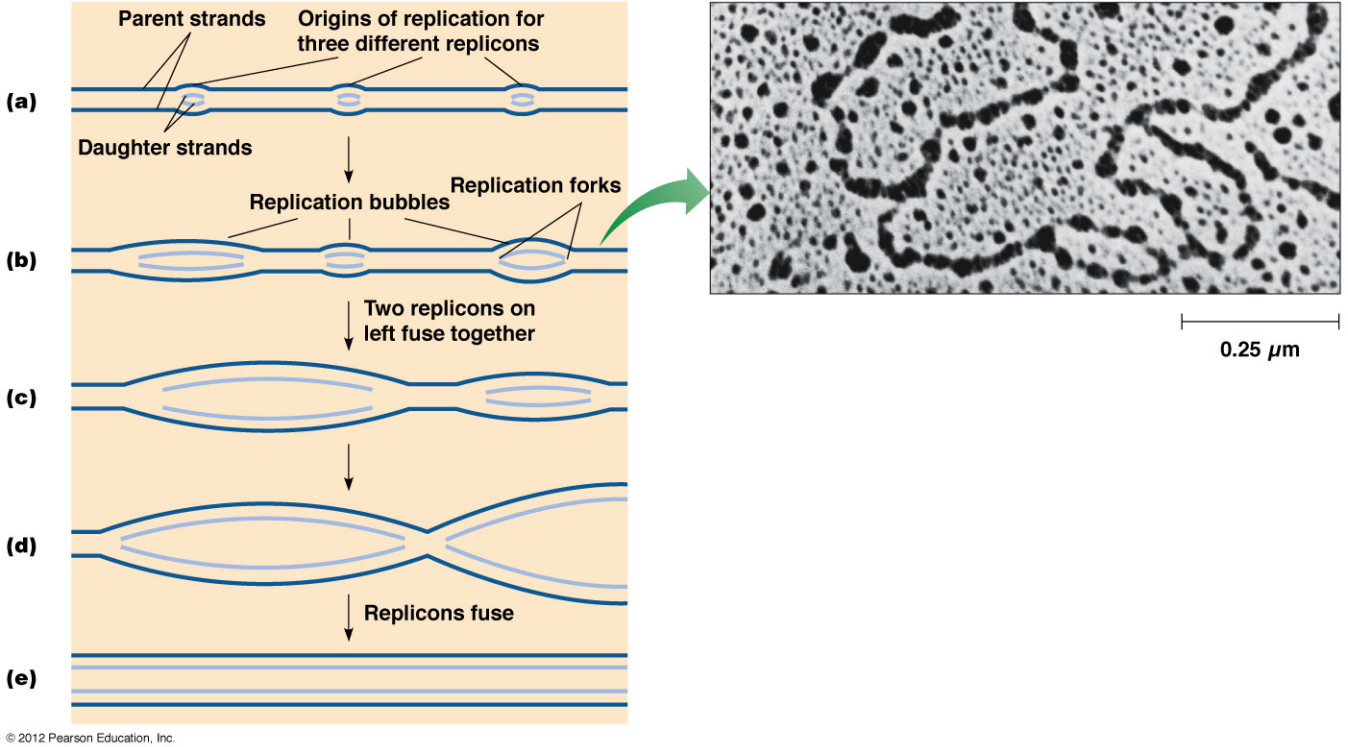
Eukaryotic DNA Replication Involves Multiple Replicons

- In eukaryotes replication of linear chromosomes is initiated at multiple sites, creating replication units called **replicons**
- At the center of each replicon is a DNA sequence called an **origin of replication**, where synthesis is initiated by several groups of *initiator proteins* (a single eukaryotic chromosome may contain several thousand replicons)
  - If eukaryotic DNA had only one origin of replication it would take about a month to duplicate a human chromosome!
- First, a multisubunit protein complex called the *origin recognition complex* (*ORC*) binds the replication origin

Eukaryotic replication (continued)

- Next, the *minichromosome maintenance* (*MCM*) proteins bind the origin
- The MCM proteins include several DNA helicases that unwind the double helix
- At this point all the DNA-bound proteins make up the **pre-replication complex** and the DNA is “licensed” for replication
- After replication begins, two replication forks synthesize DNA in opposite directions, forming a replication bubble that grows as replication proceeds

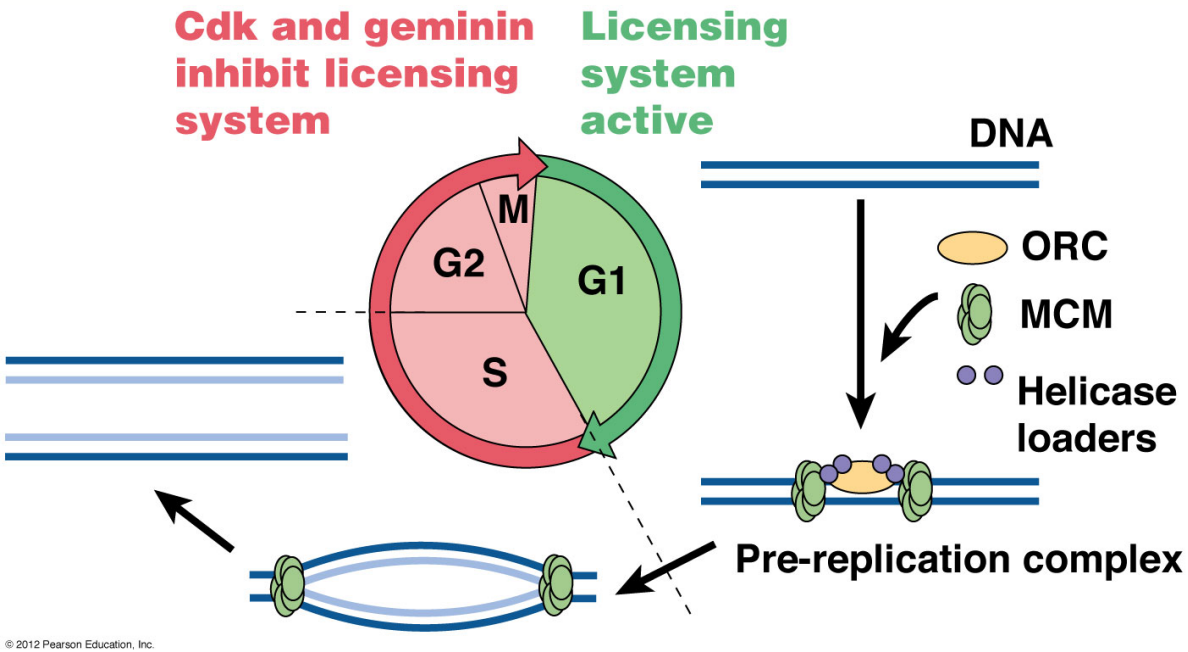
Figure 19-5A-E



Replication Licensing Ensures That DNA Molecules Are Duplicated Only Once Prior to Each Cell Division

- Licensing is provided by binding of MCM proteins to the origin, which requires both ORC and helicase loaders
- It ensures that after DNA is replicated at each origin, the DNA cannot be licensed for replication again until after mitosis
- After replication begins, the MCM proteins are removed from the origins and cannot bind again (due to Cdk and geminin binding)

Figure 19-6


Cdk and geminin inhibit licensing system
Licensing system active
DNA
M
G2
G1
S
ORC
MCM
Helicase loaders
Pre-replication complex
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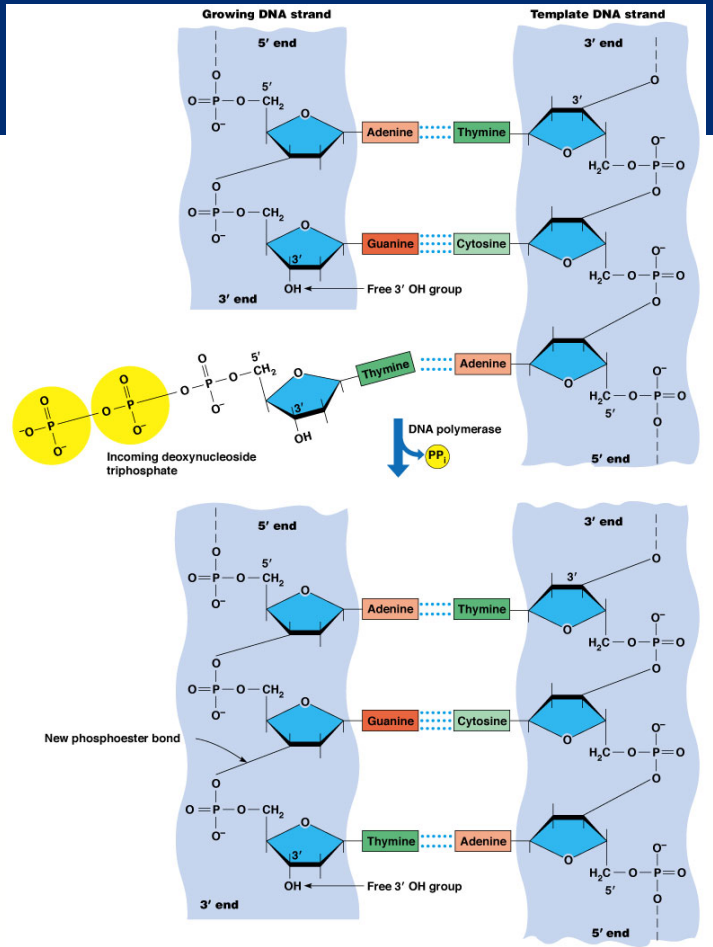
DNA Polymerases Catalyze the Elongation of DNA Chains

- **DNA polymerase** is an enzyme that can copy DNA molecules
- Incoming nucleotides are added to the 3′ hydroxyl end of the growing DNA chain, so elongation occurs in the **5′ to 3′ direction**
- Several forms of DNA polymerase have been identified; the original is now called DNA polymerase I

Figure 19-7

The Directionality of DNA Synthesis:

DNA polymerase catalyzes the addition of deoxynucleoside triphosphate (5’ to 3’)

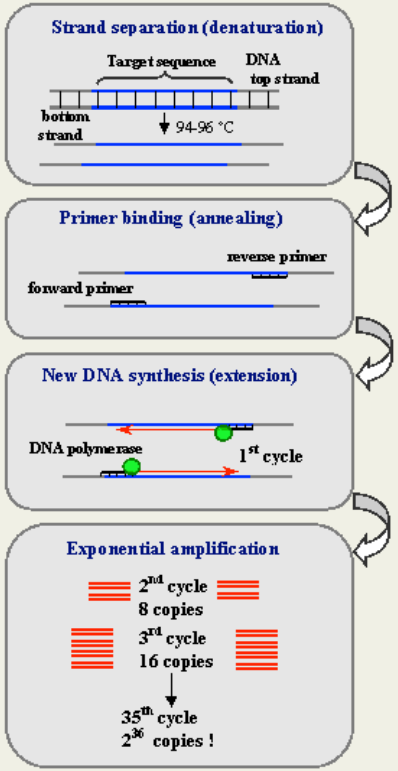


Biotechnology functions of DNA polymerases

- DNA polymerases have practical applications in biotechnology
- The *polymerase chain reaction* is a technique in which a DNA polymerase is used to amplify tiny samples of DNA

How It Works

https://www.dnalc.org/resources/3d/19-polymerase-chain-reaction.html



www.ncbi.nlm.nih.gov

The PCR reaction requires the following components:

DNA template - the sample DNA that contains the target sequence. At the beginning of the reaction, high temperature is applied to the original double-stranded DNA molecule to separate the strands from each other.

DNA polymerase - a type of enzyme that synthesizes new strands of DNA complementary to the target sequence. The first and most commonly used of these enzymes is *Taq* DNA polymerase (from *Thermis aquaticus*), whereas *Pfu* DNA polymerase (from *Pyrococcus furiosus*) is used widely because of its higher fidelity when copying DNA. Although these enzymes are subtly different, they both have two capabilities that make them suitable for PCR: 1) they can generate new strands of DNA using a DNA template and primers, and 2) they are heat resistant.

Primers - short pieces of single-stranded DNA that are complementary to the target sequence. The polymerase begins synthesizing new DNA from the end of the primer.

Nucleotides (dNTPs or deoxynucleotide triphosphates) - single units of the bases A, T, G, and C, which are essentially "building blocks" for new DNA strands.

RT-PCR (**R**everse **T**ranscription **PCR**) is PCR preceded with conversion of sample RNA into cDNA with enzyme **reverse transcriptase** .

Applications of PCR:

cloning, genetic engineering, sequencing

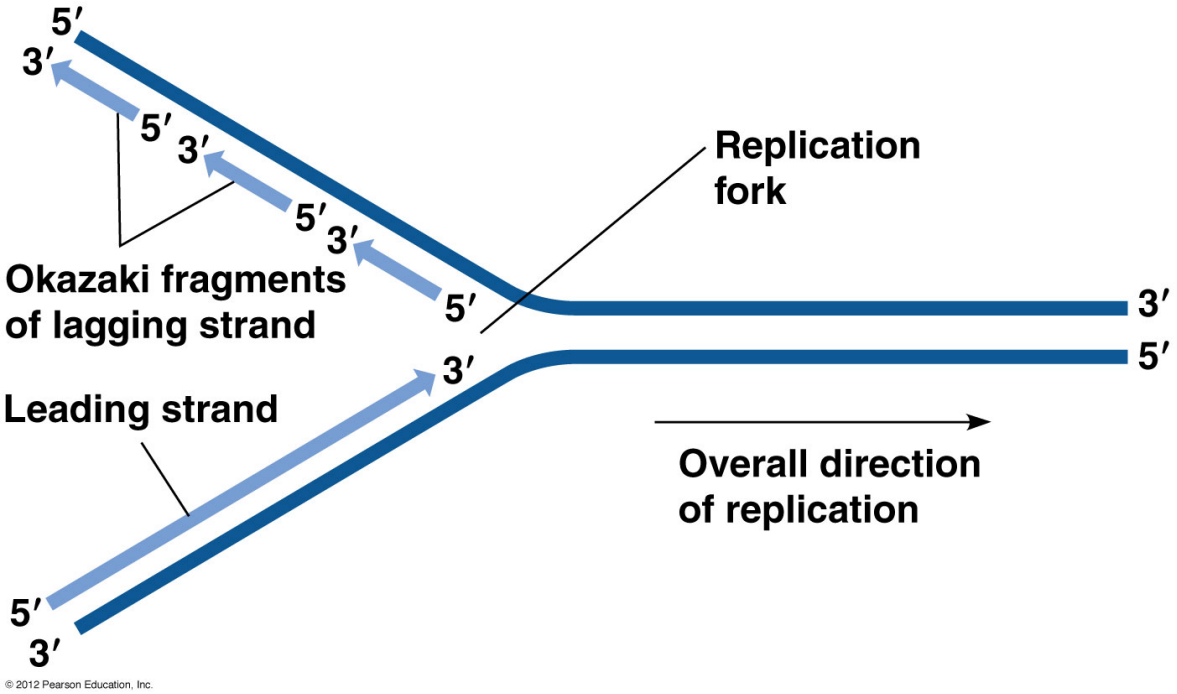
Limitations of PCR and RT-PCR:

The PCR reaction starts to generate copies of the target sequence exponentially. Only during the exponential phase of the PCR reaction is it possible to extrapolate back to determine the starting quantity of the target sequence contained in the sample. Because of inhibitors of the polymerase reaction found in the sample, reagent limitation, accumulation of pyrophosphate molecules, and self-annealing of the accumulating product, the PCR reaction eventually ceases to amplify target sequence at an exponential rate and a "plateau effect" occurs, making the end point quantification of PCR products unreliable. This is the attribute of PCR that makes Real-Time Quantitative RT-PCR so necessary.

DNA Is Synthesized as Discontinuous Segments That Are Joined Together by DNA Ligase

- DNA is synthesized in the 5′ to 3′ direction, but the two strands of the double helix are oriented in opposite directions
- One strand (the **lagging strand**) is synthesized in discontinuous fragments called **Okazaki fragments**
  - These are then joined by DNA ligase to form a continuous new 3′ to 5′ DNA strand
- The other (the **leading strand**) is synthesized in a continuous chain

Figure 19-9



- Replication
- Leading strand/lagging strand
- Okazaki fragments

https://www.youtube.com/watch?v=TEQMeP9GG6M

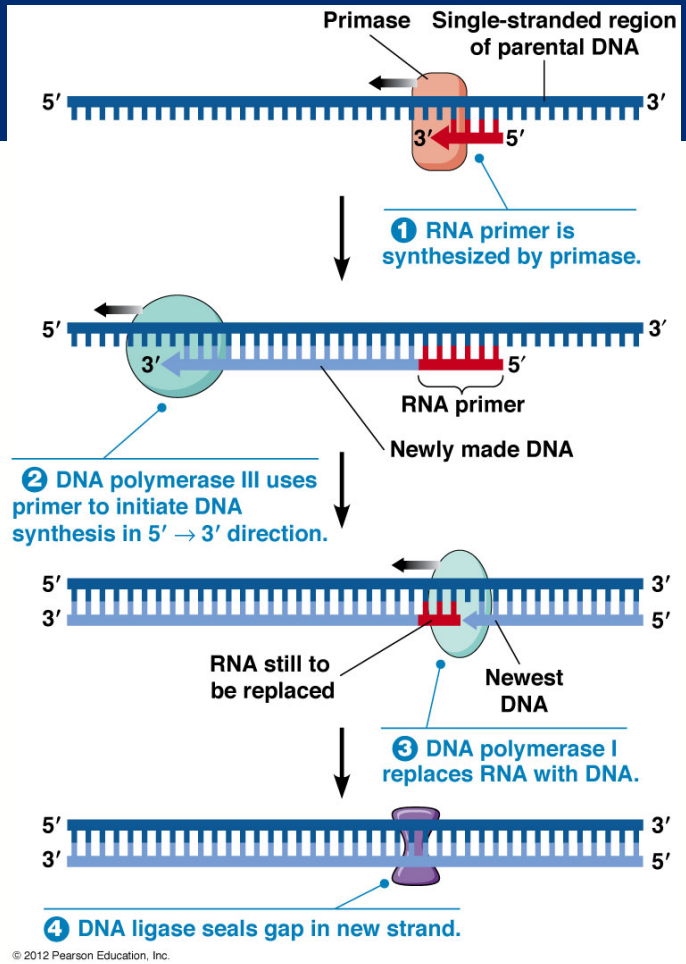
Proofreading Is Performed by the 3′→ 5′ Exonuclease Activity of DNA Polymerase

- About 1 of every 100,000 nucleotides incorporated during DNA replication is incorrect
- Such mistakes are usually fixed by a **proofreading mechanism**
- Almost all DNA polymerases have a 3′ → 5′ exonuclease activity
- **Exonucleases** degrade nucleic acids from the ends of the molecules; **Endonucleases** make internal cuts (vital for repair)
  - The exonuclease activity of DNA polymerase allows it to remove incorrectly base-paired nucleotides and incorporate the correct base

DNA synthesis requires RNA primers

- Natural DNA synthesis (not PCR) is initiated by the formation of short RNA primers

- These are synthesized by primase using a single DNA strand as the template



The process of DNA synthesis

- Once the RNA primer is made, a DNA polymerase III adds deoxynucleotides (A's, C's, T's, or G's) to the 3′ end of the primer

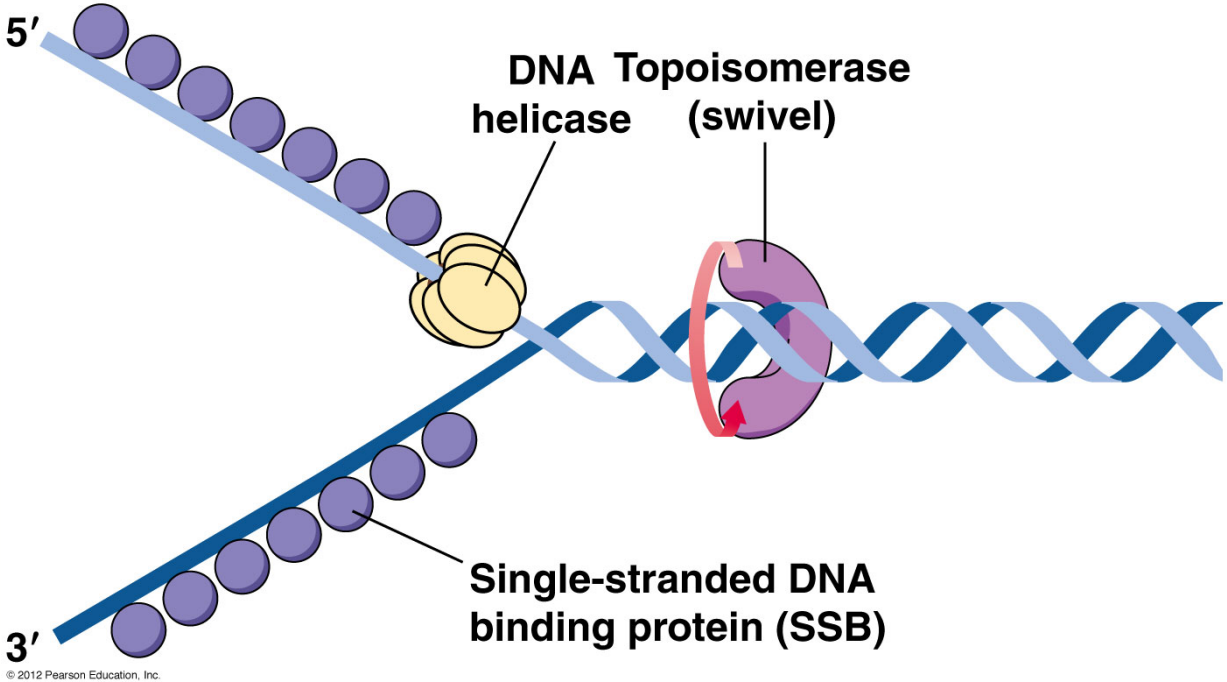
- For the leading strand, just one primer is needed, but the lagging strand needs a series of primers to initiate each Okazaki fragment

- When the DNA chain reaches the next Okazaki fragment the RNA is degraded and replaced with DNA; adjacent fragments are joined together by DNA ligase

Unwinding the DNA Double Helix Requires DNA Helicases, Topoisomerases, and Single-Stranded DNA Binding Proteins

- During DNA replication the two strands of the double helix must unwind at each replication fork
- Three classes of proteins facilitate the unwinding: *DNA helicases*, *topoisomerases*, and *single-stranded DNA binding proteins (SSB)*
  - **DNA helicases** are responsible for unwinding the DNA, using energy from ATP hydrolysis
  - **Topoisomerases** create swivel points in the DNA molecule by making and then quickly sealing double-strand or single-stranded breaks (e.g. gyrase)
  - **SSB** keep the DNA unwound and accessible to the replication machinery

Figure 19-12



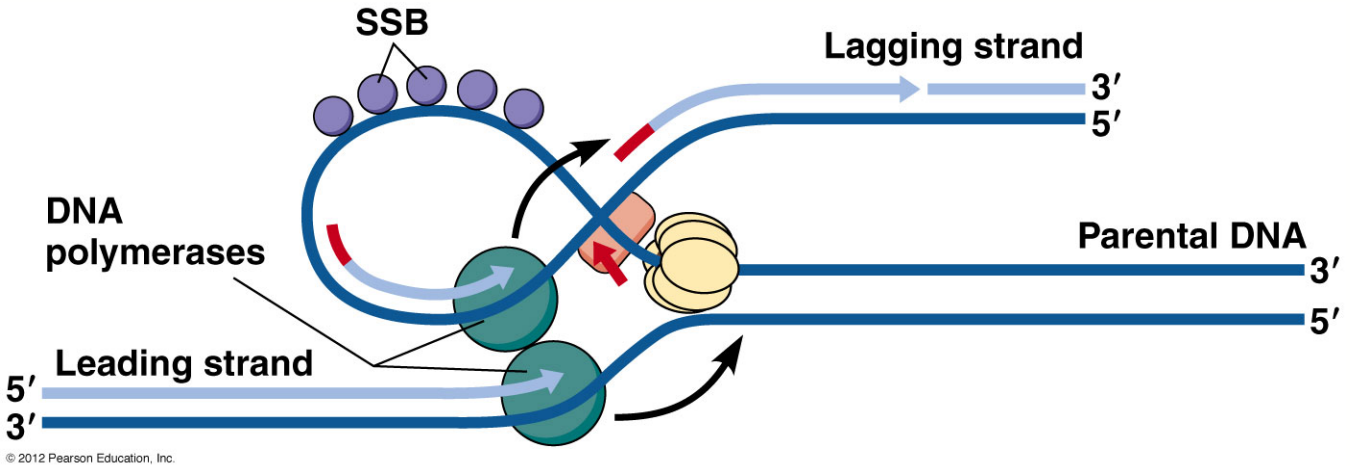
Summary: DNA Replication

- Starting at the origin of replication, the machinery at the replication fork adds proteins required for synthesizing DNA

- These are DNA helicase, DNA gyrase, SSB, primase, DNA polymerase, and DNA ligase

- Several other proteins are used to improve the efficiency, e.g., a ring-shaped *sliding clamp* keeps DNA polymerase firmly attached to DNA

Figure 19-14


SSB
Lagging strand
3′
5′
DNA
polymerases
Parental DNA
3′
5′
Leading strand
5′
3′
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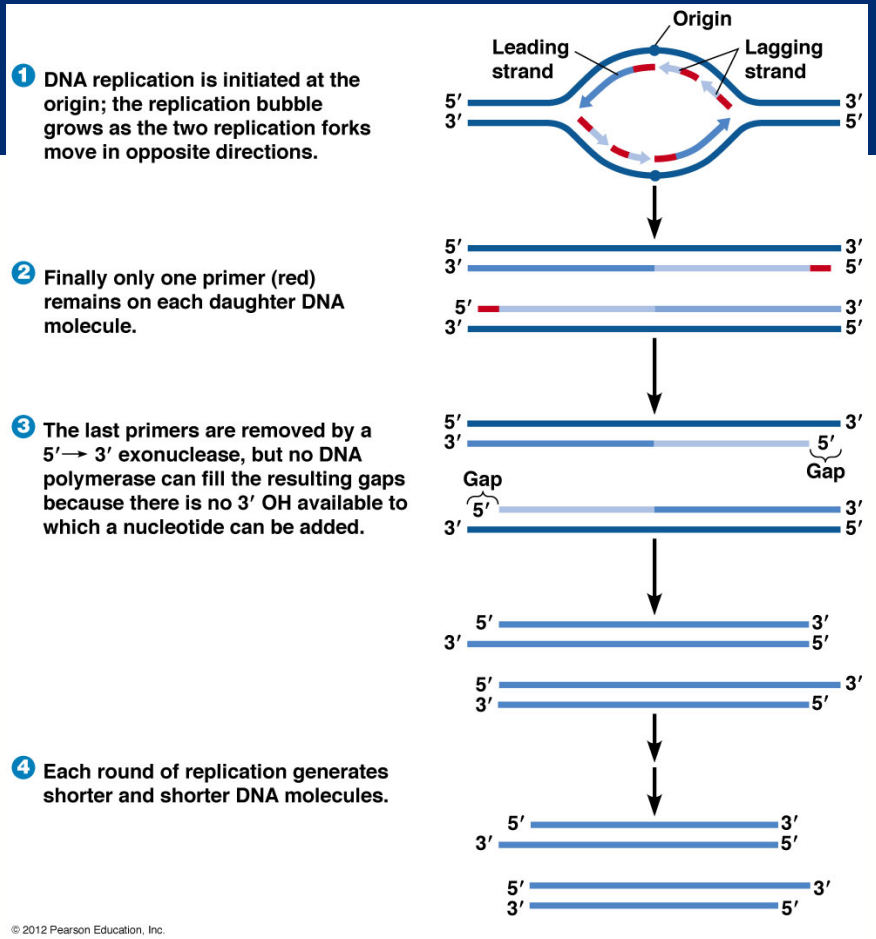
DNA biosynthesis summary (in real time):

http://www.youtube.com/watch?v=I9ArIJWYZHI

Telomeres Solve the DNA End-Replication Problem

- Linear DNA molecules have a problem in completing DNA replication on the lagging strand, because primers are required
- Each round of replication would end with the loss of some nucleotides from the ends of each linear molecule
- Eukaryotes solve this problems with **telomeres**, highly repeated sequences at the ends of chromosomes

Figure 19-15



Telomeres and telomerase

- Human telomeres have 100 to 1500 copies of TTAGGG at the ends of chromosomes
- These noncoding sequences ensure that the cell will not lose important genetic information if DNA molecules shorten during replication
- A polymerase called **telomerase** can catalyze the addition of repeats to chromosome ends (to lengthen the telomere)
- In multicellular organisms telomerase function is restricted to *germ cells* and a few other types of actively proliferating cells

Most cells have a limited life span

- Telomere shortening occurs with each cell division in most cells

- As a result, telomere length is a sort of counting device for how many times a cell has divided; if a cell divides too many times, telomeres could be lost

- Cells at risk of loss of telomeres undergo *apoptosis*, programmed cell death

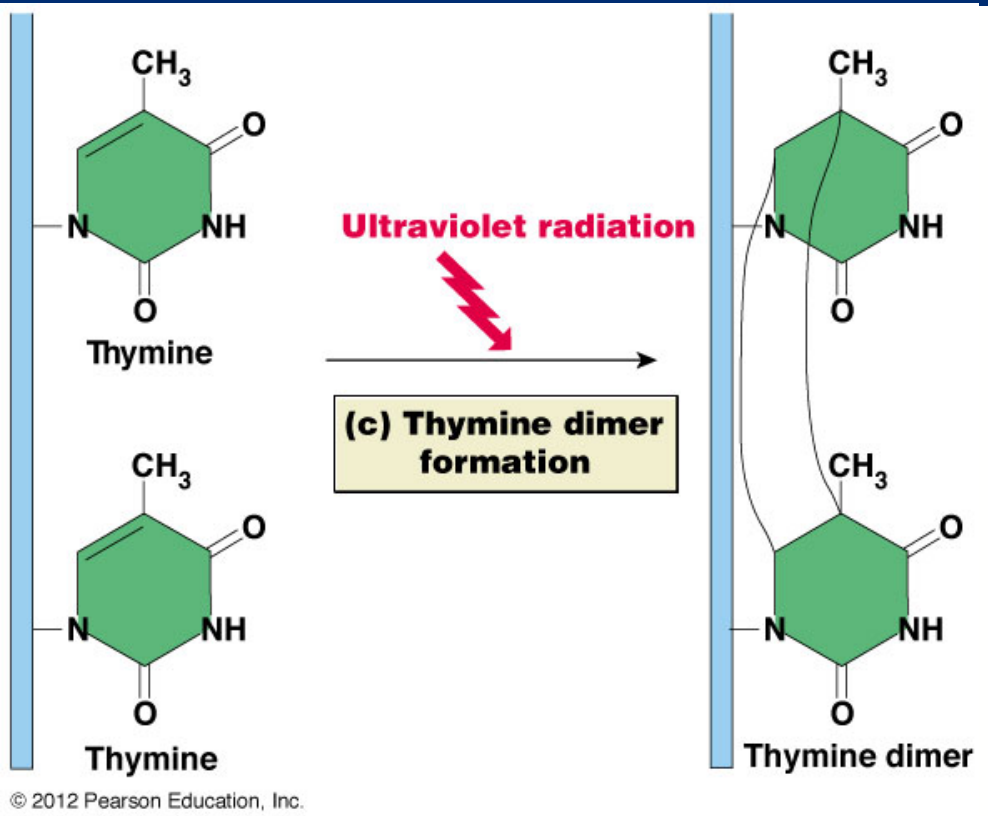
DNA Damage and Repair

- DNA must be accurately passed on to daughter cells
- In addition to ensuring that replication is faithful, this also means that DNA alterations must be repaired
- DNA alterations, or **mutations**, can arise spontaneously, or through exposure to environmental agents

DNA Damage Can Occur Spontaneously or in Response to Mutagens

- During DNA replication, some types of mutations occur through spontaneous hydrolysis reactions
  - *Depurination* is loss of a purine base (A or G)
  - *Deamination* is removal of a base's amino group, changing its base-pairing properties
- DNA damage can be caused by mutation-causing agents, **mutagens**
  - Environmental mutagens fall into two categories: chemicals and radiation
  - Mutagenic chemicals alter DNA structure through a variety of mechanisms

Figure 19-17C



Translesion Synthesis and Excision Repair Correct Mutations Involving Abnormal Nucleotides

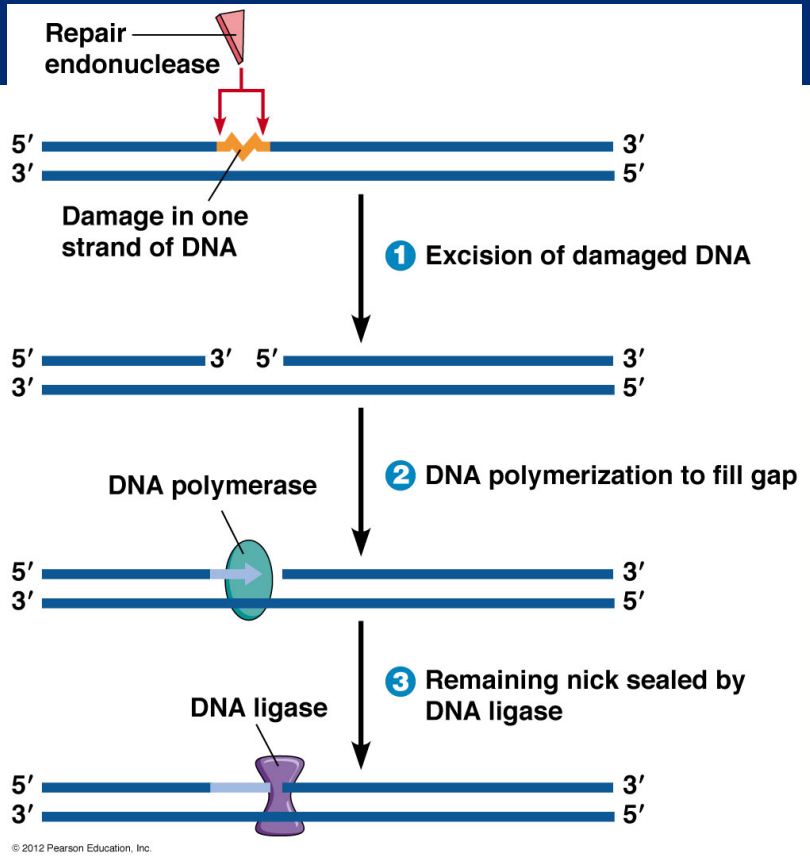
- **A variety of mechanisms are used for DNA repair**

- Repair during replication
  - Involves specialized DNA polymerases that carry out **translesion synthesis**

- Repairs after DNA replication
  - Called **excision repair**, in which abnormal nucleotides are removed and replaced
    - *E. coli* has nearly 100 genes that code for proteins involved in this process
    - Excision repair works by a basic three-step process

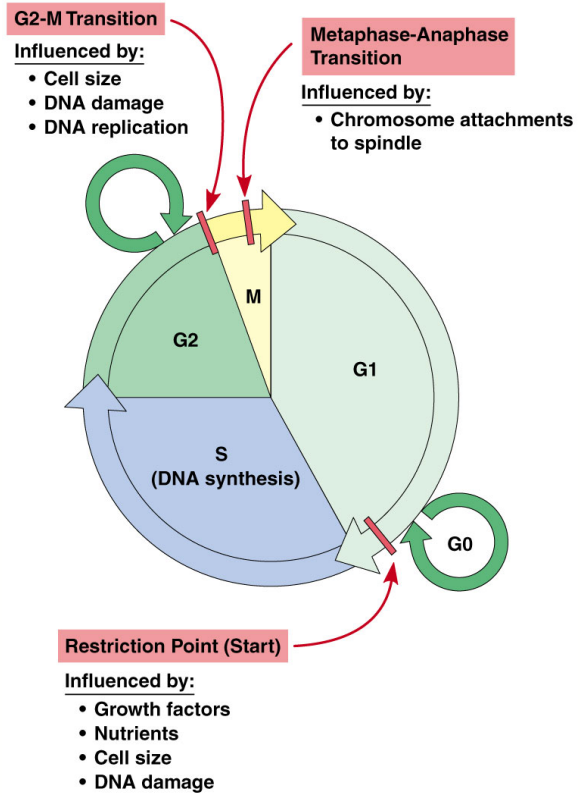
Excision repair

Repair endonucleases **are recruited to DNA by proteins that recognize damage**



Progression Through the Cell Cycle Is Controlled at Several Key Transition Points

- Control of the cell cycle must:
  - 1. Ensure that events of each phase are carried out in the correct order and at the appropriate time
  - 2. Ensure that each phase is completed before the next one begins
  - 3. Respond to external conditions



Checkpoint Pathways Monitor Chromosome-to-Spindle Attachments, Completion of DNA Replication, and DNA Damage

- If cells proceeded from one phase of the cell cycle to the next without completing each step, daughter cells might be abnormal
  - E.g., **aneuploidy** (incorrect number of chromosomes) could result
- Cells use a series of checkpoints that ensure each phase is completed properly before the next one begins

Checkpoints

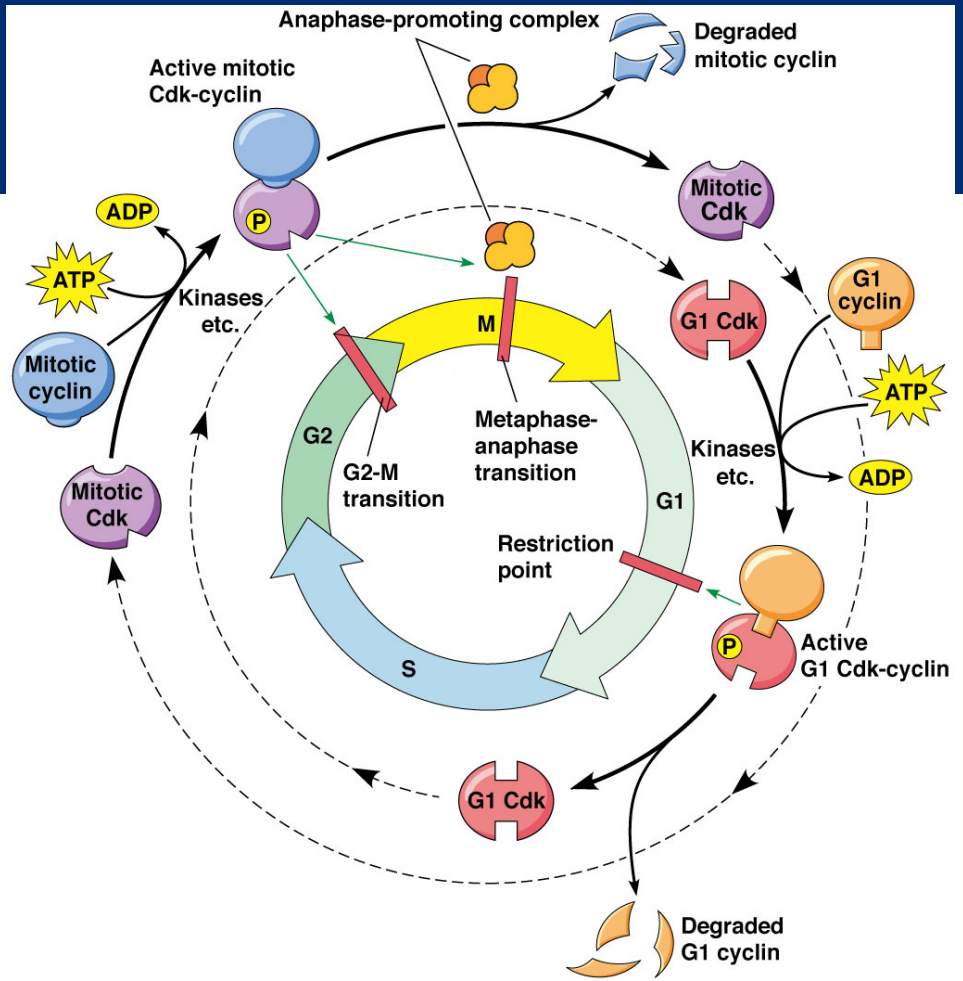
- The **mitotic spindle checkpoint** prevents anaphase from beginning before the chromosomes are all attached to the spindle

- The **DNA replication checkpoint** ensures that DNA synthesis is complete before the cell begins mitosis

- A multiple series of **DNA damage checkpoints** monitor DNA for damage and halt the cell cycle at various points
  - **p53** protein, the "guardian of the genome," plays a central role in these checkpoint pathways
    - Regulator of cell cycle, and therefore tumor suppressor
    - Classified as a "tumor suppressor gene" by Bert Vogelstein at JHMI in 1989
    - p53 stimulates production of enzymes involved in DNA repair
    - But if the damage cannot be repaired, p53 activates genes needed to trigger cell death by apoptosis

Figure 19-41

Summary: The Cell Cycle Regulation Machine



Apoptosis

- Damaged or diseased cells need to be eliminated
- In such cases, the process must not damage surrounding cells
- Multicellular organisms accomplish this through a programmed cell death—**apoptosis**
- Apoptosis proceeds through the activation of a series of enzymes called **caspases**

Necrosis

- Cell death called necrosis sometimes follows tissue injury

- Necrosis involves swelling and rupture of injured cells, whereas apoptosis involves a specific series of events that lead to dismantling of the cell contents

Apoptosis Is Triggered by Death Signals or Withdrawal of Survival Factors

There are two main routes by which cells can activate caspases and enter apoptosis:

- Activation can occur directly, e.g., when human cells are infected by viruses, *cytotoxic T lymphocytes* are activated and induce apoptosis. This is triggered when cells receive *cell death signals*

- 2. When **survival factors** are withdrawn, a cell may enter apoptosis
  - The site of action is the mitochondrion
  - Healthy cells have several *anti-apoptotic* proteins in the outer mitochondrial membrane

Damaged cells can trigger their own apoptosis

- If a cell suffers such damage that it can't repair itself, it may trigger its own demise

- It can enter apoptosis through the activity of p53

How could this natural process be used to treat cancer?

Summary

- DNA Replication
- DNA Damage and Repair
- Cell Checkpoints
- Cell Death