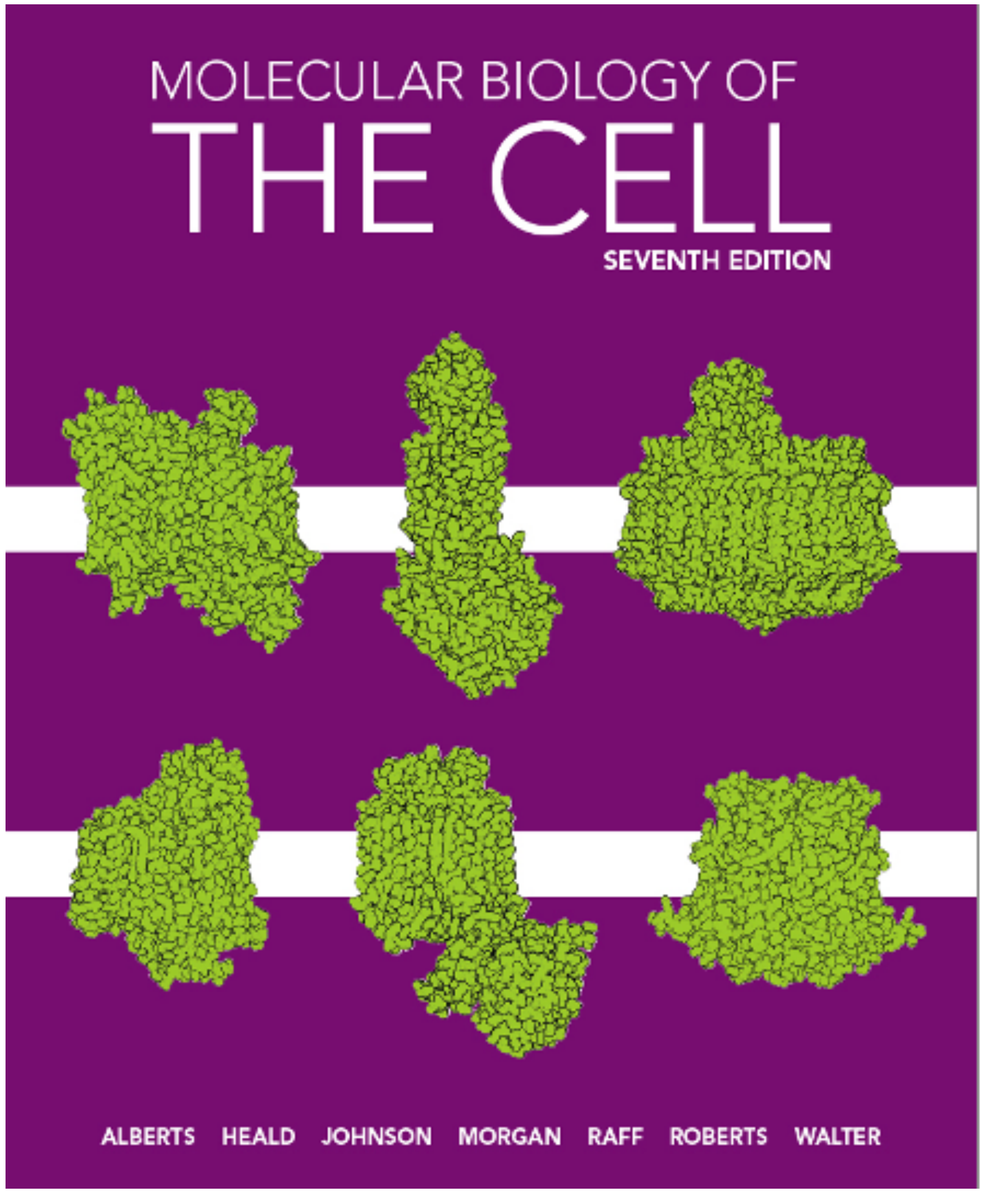
Cellular and Molecular Biology I

BIO-205-4

Camille Goemans



Chapter 5

DNA Replication, Repair, and Recombination

Plan

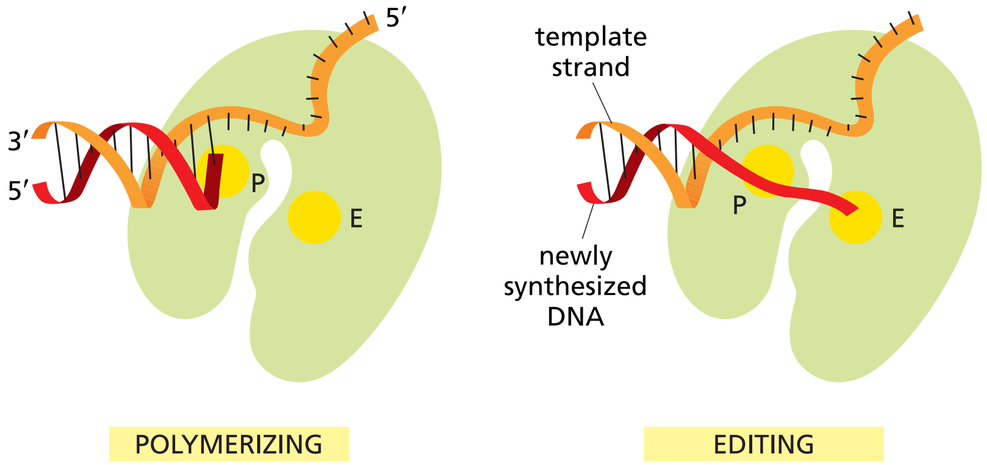
- Quick recap
- DNA replication mechanisms
- The initiation and completion of DNA replication in chromosomes
- DNA repair
- Transposition and conservative site-specific recombination

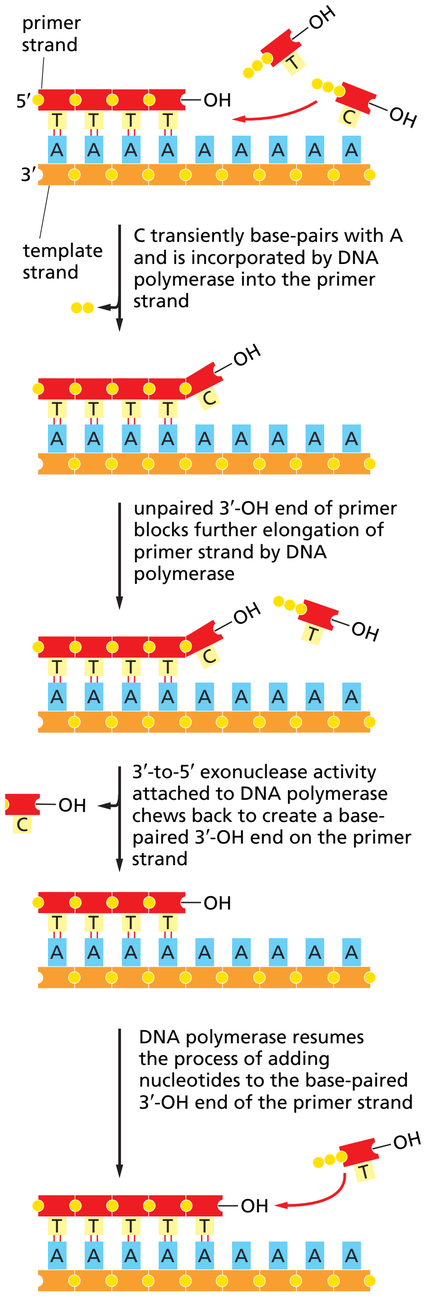
Difficulties associated with DNA replications

1. Errors

- Step 1 - **Proofreading by DNA polymerase**, incorrect nucleotides are harder to add to the chain (energetically less favorable) and are more likely to diffuse away

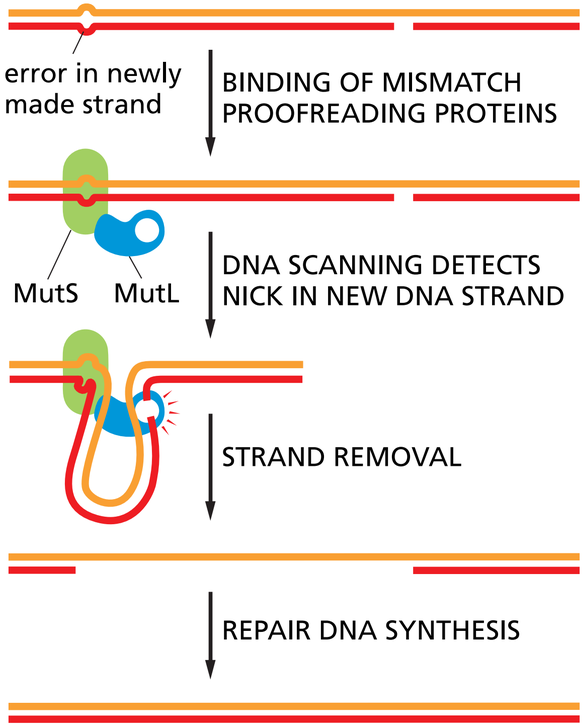
- Step 2- **Exonucleic proofreading by DNA polymerase** takes place when an incorrect nucleotide is inserted





1. Errors

- Step 3- **Strand-directed mismatch repair** detects distortion into mismatched DNA
- It needs to recognise the **new strand**
- In *E. coli*, this depends on the **methylation status** of all adenines on the DNA (which takes some time)
- Similar system in Eukaryotes, that recognises **nicks in the lagging strand** before the action of the DNA ligase - not clear how it works on the leading strand



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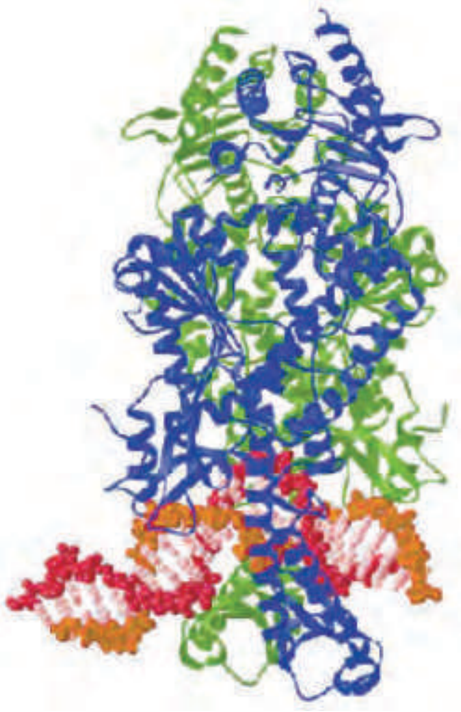
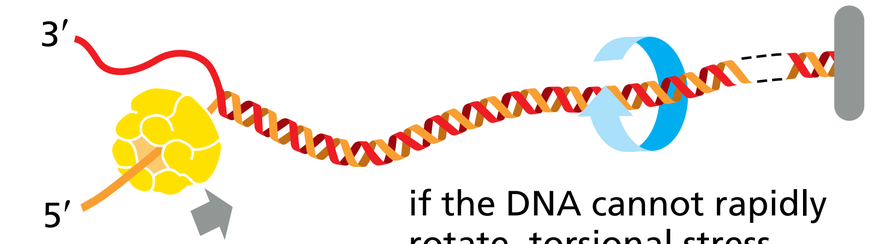


(B)

2. Preventing DNA tangling

- For replication to occur, the double helix needs to **unwind** ahead of the replication fork (which is energetically unfavourable)

used as fair quote from
http://www.biochem.arizona.edu/classes/bioc462/462a/NOTES/Nucleic_Acids/Fig24_11SupercoilStrandSep.GIF



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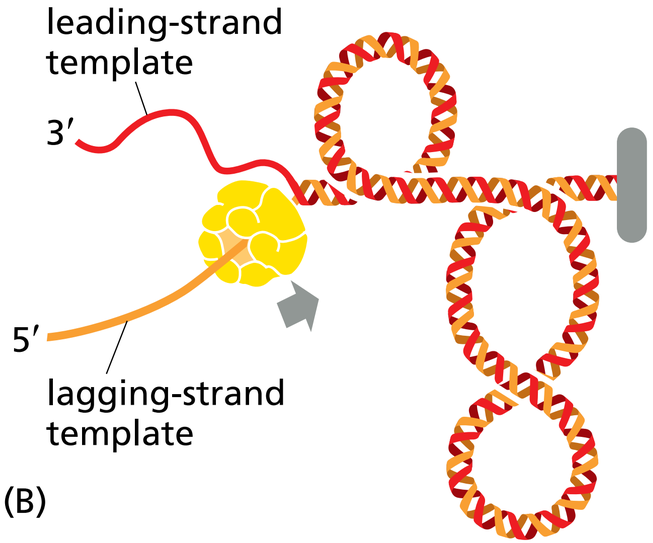

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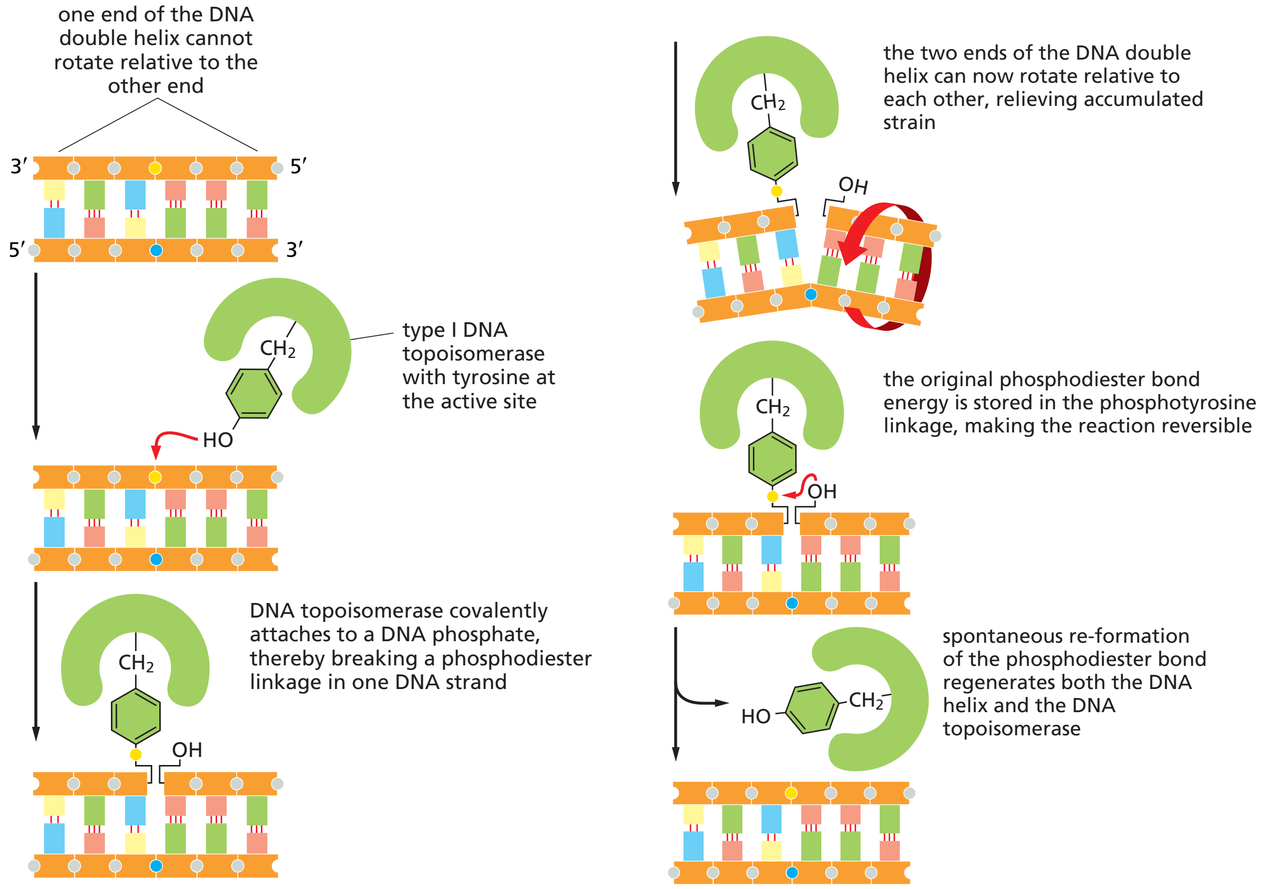
Preventing DNA tangling

- The overwinding is relieved by proteins known as **DNA topoisomerases**
- They have different **mechanisms of action** (one example here)



Eukaryotes vs. Prokaryotes

- Most of what we know about replication derives from **studies in bacteria and bacteriophages** (viruses that infect bacteria)
- Most mechanisms have been **conserved through evolution**
- The mechanisms are typically **more complex** in Eukaryotes

Replication actors

Test - Who is doing what?

- DNA polymerase
- Primase
- Ligase
- Helicase
- ssDNA binding proteins
- Sliding clamp
- DNA topoisomerase

Plan

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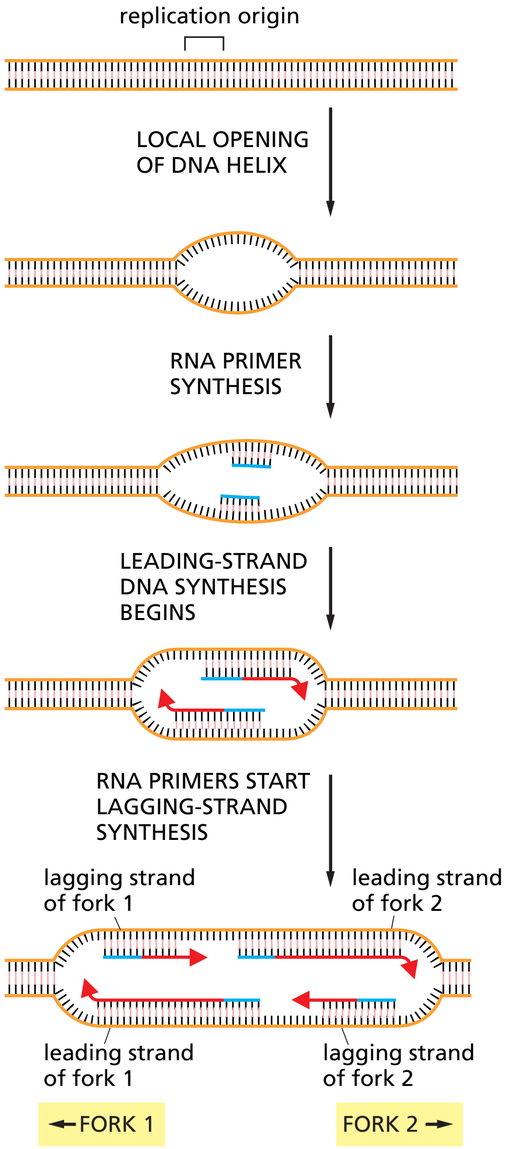
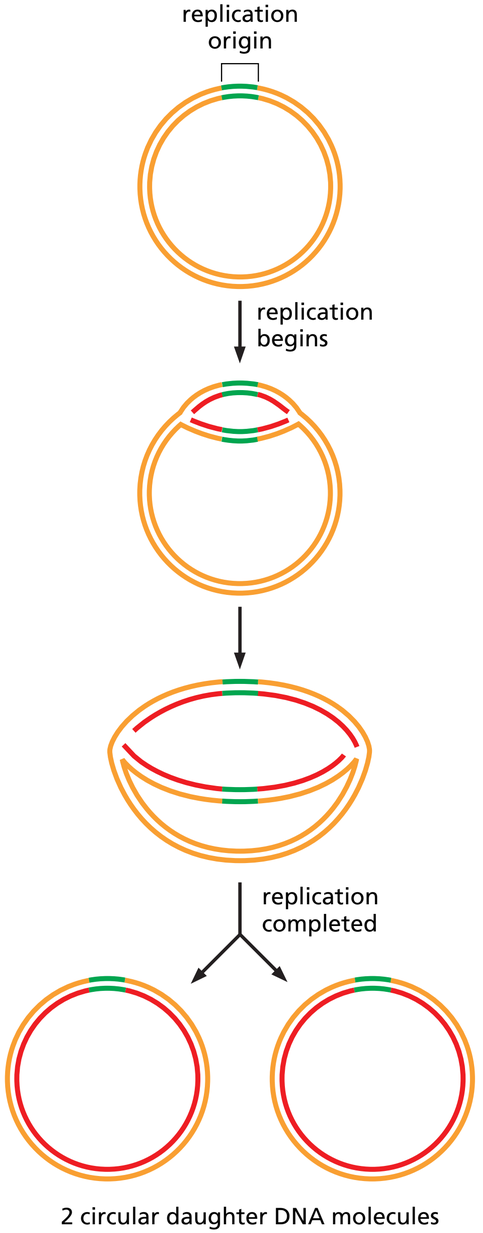
DNA synthesis begins at replication origins

- How does the whole process **start**?
- DNA double helix is **very stable**
- **Initiator proteins** bind to **replication origins** (more AT-rich sequences -held by less hydrogen bonds) and open the two strands by breaking the hydrogen bonds
- Those are specific **DNA sequences** that attract initiator proteins



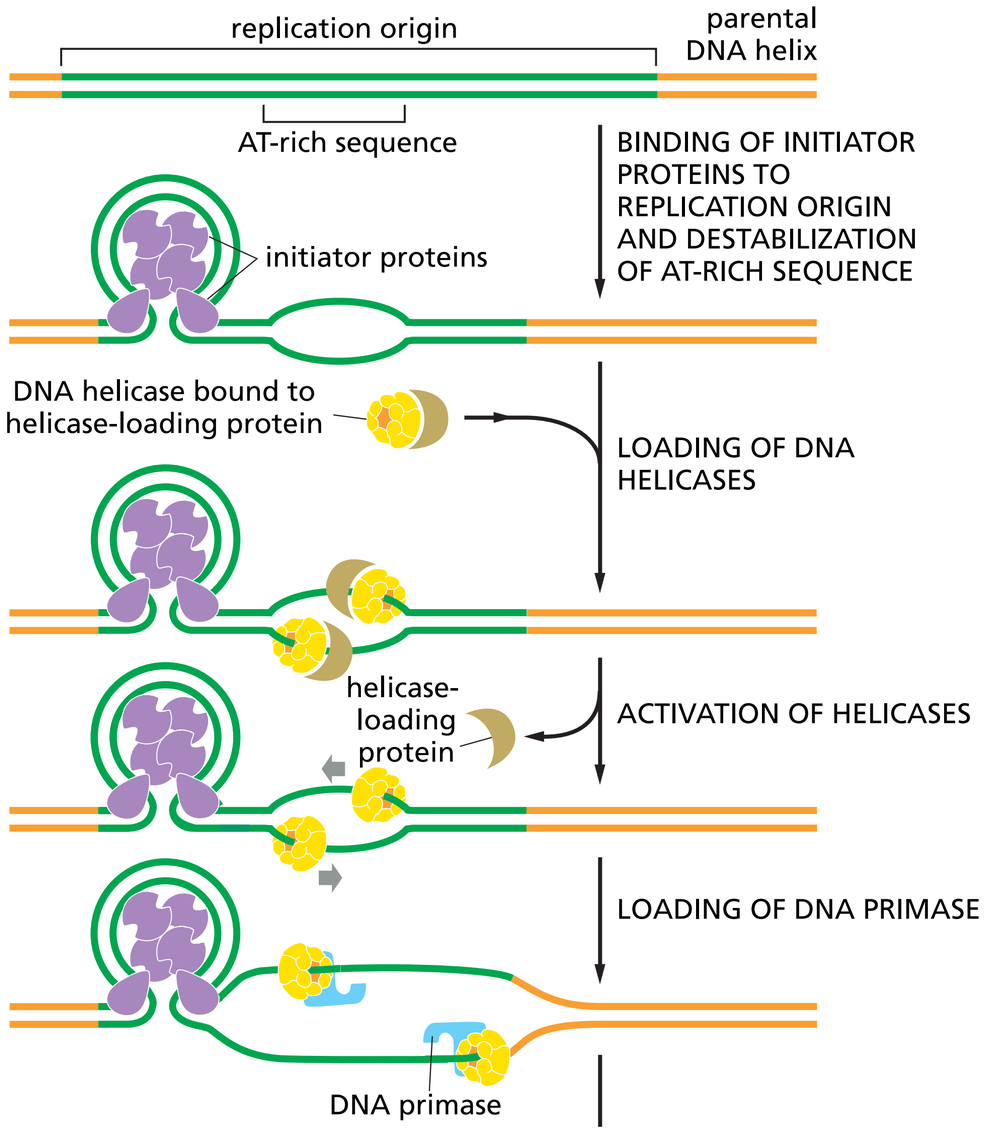
In bacteria

- Reminder: bacteria typically have **one circular chromosome**
- Replication starts at **<u>one</u> origin of replication**
- Initiation of replication is **tightly regulated**
- Because they are growing **fast,** bacteria replicate their DNA **“continuously”**



In bacteria

1. **Initiator proteins** bind to the origin of replication
2. The helix is **destabilized**
3. **DNA helicases** are recruited
4. **DNA primase** synthesizes the primers

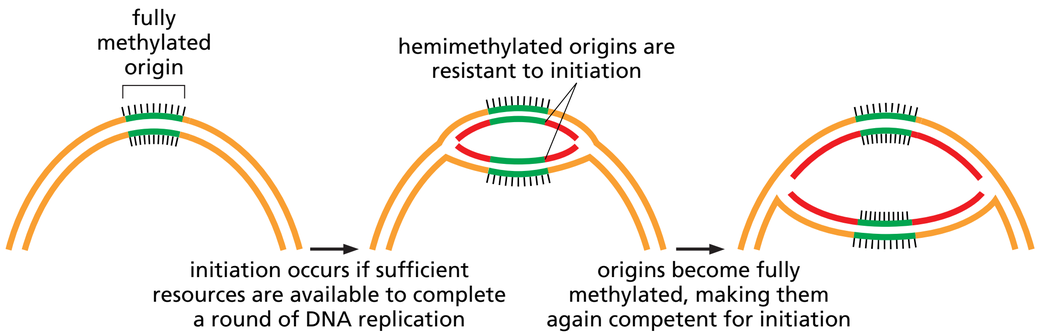


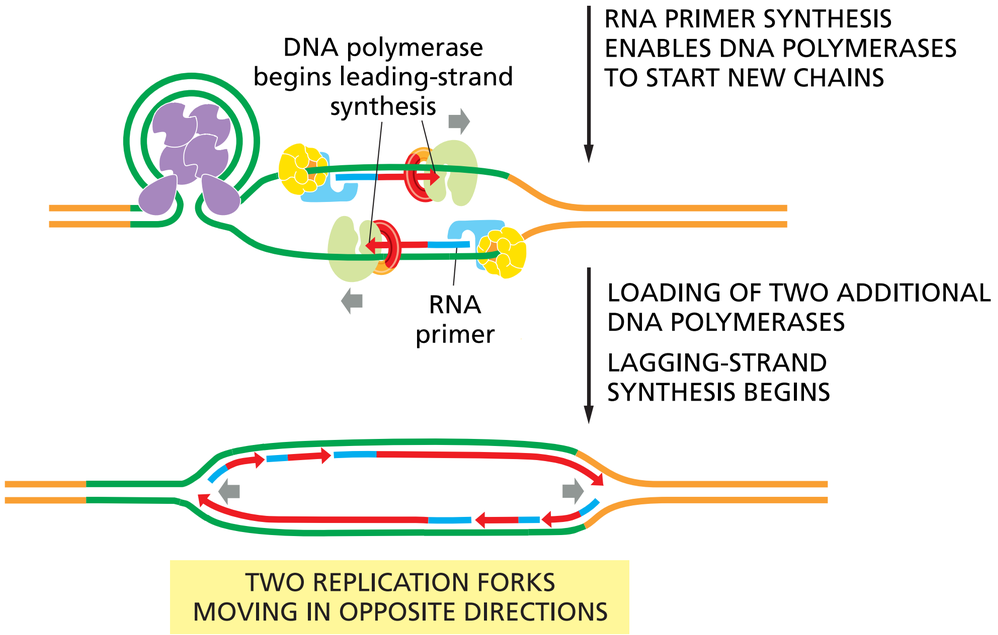
In bacteria

5. Creation of **<u>two</u> replication forks** that move in opposite directions

6. **Inactivation** of the initiator proteins

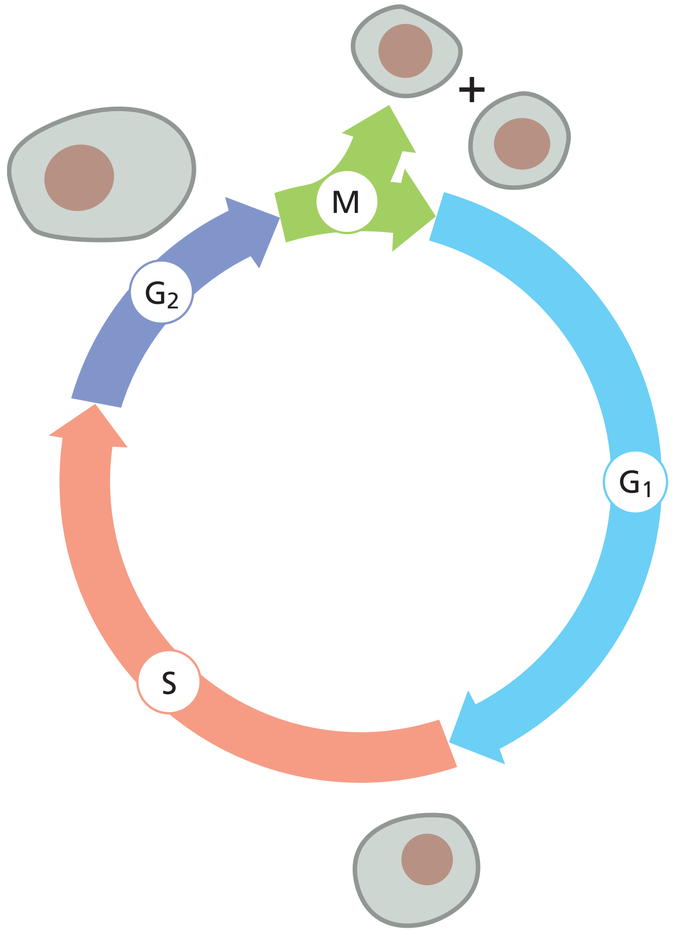
7. **Refractory period** until origin is methylated





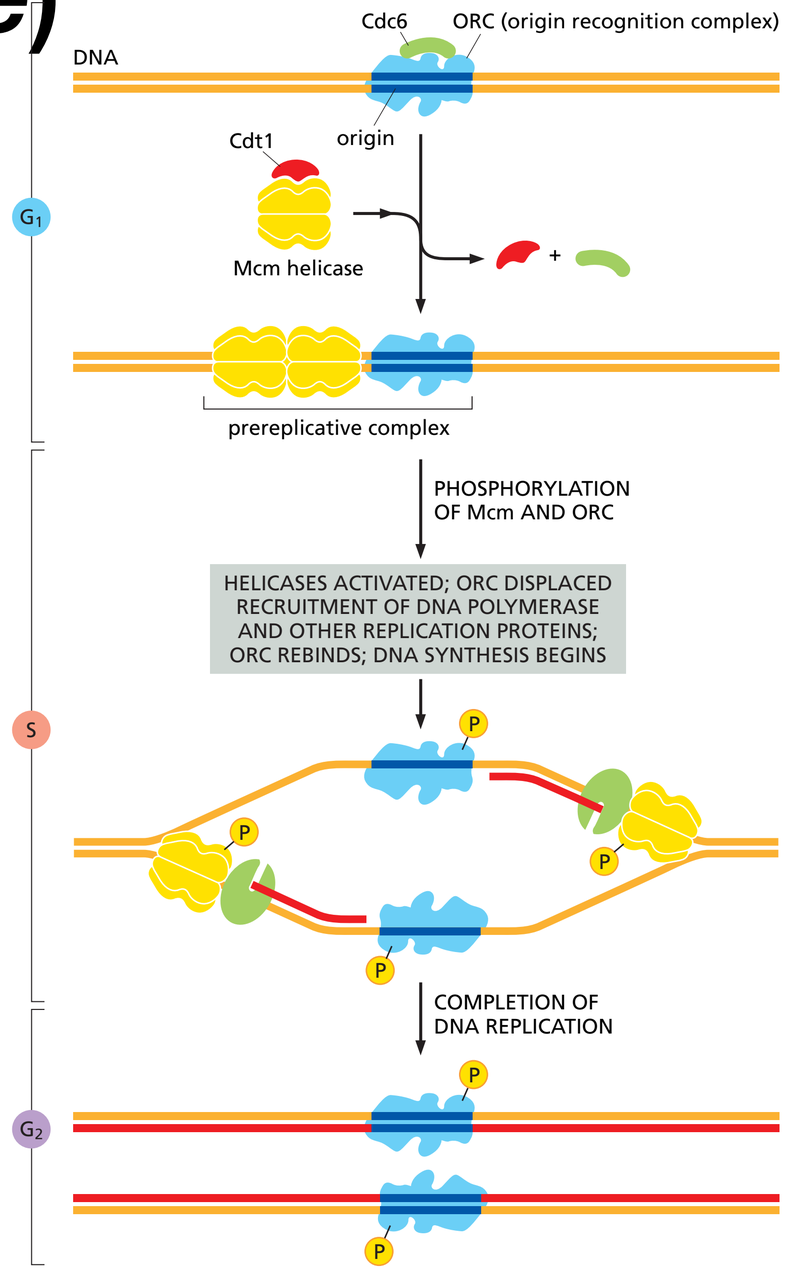
In eukaryotes

- **Multiple origins of replication** (~30 000 to ~50 000 for each cell division)
- The human genome has **more potential origins** than this; **different cells** use different origins
- **Two replication forks** are created at each origin and stop when they reach **another one** going in the opposite direction, or when they reach **the end of a chromosome**
- Replication only occurs during the **S-phase** of the cell cycle (~8h in mammalian cells)



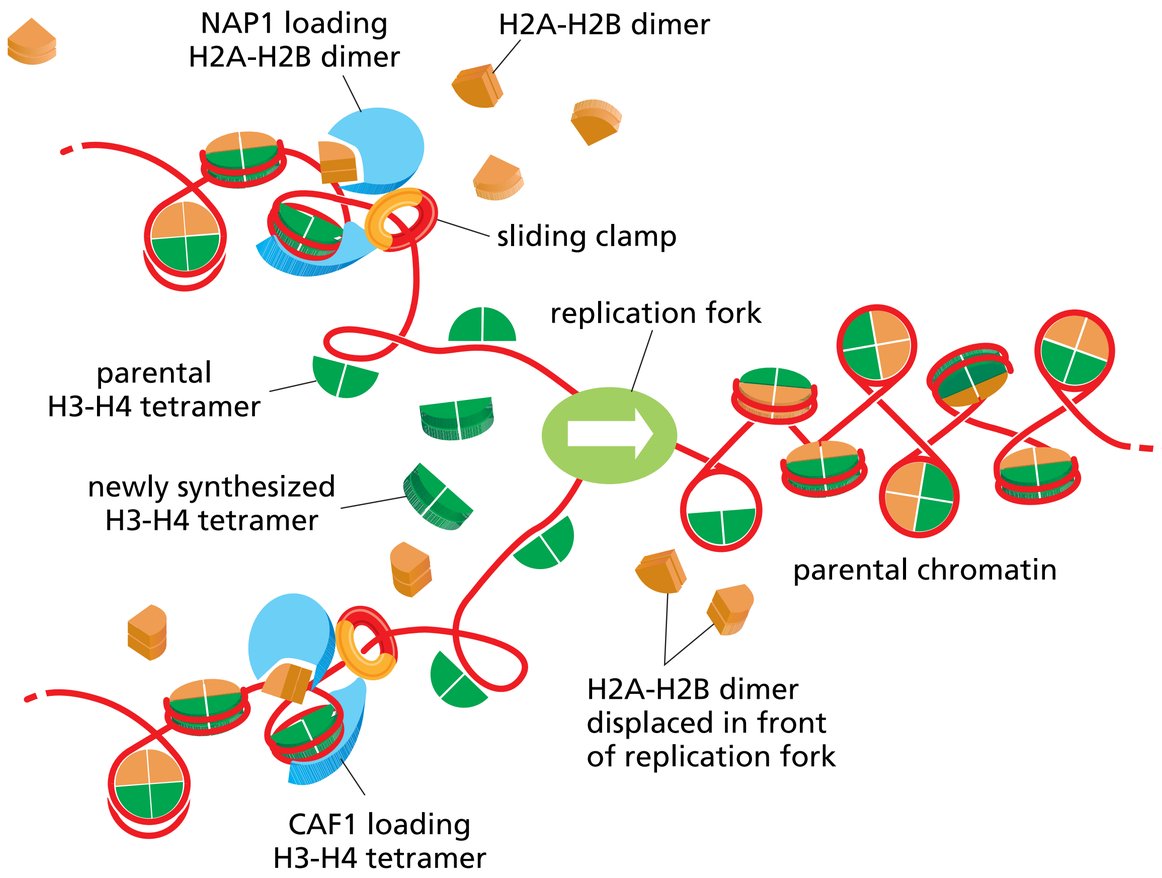
In eukaryotes (ex: *S. cerevisiae*)

- Binding of a large multi-subunit initiator protein called **ORC**
- **Phosphorylation of ORC by specific protein kinases** to prevent it from accepting other helicases
- This ensures only **one replication per cell cycle**



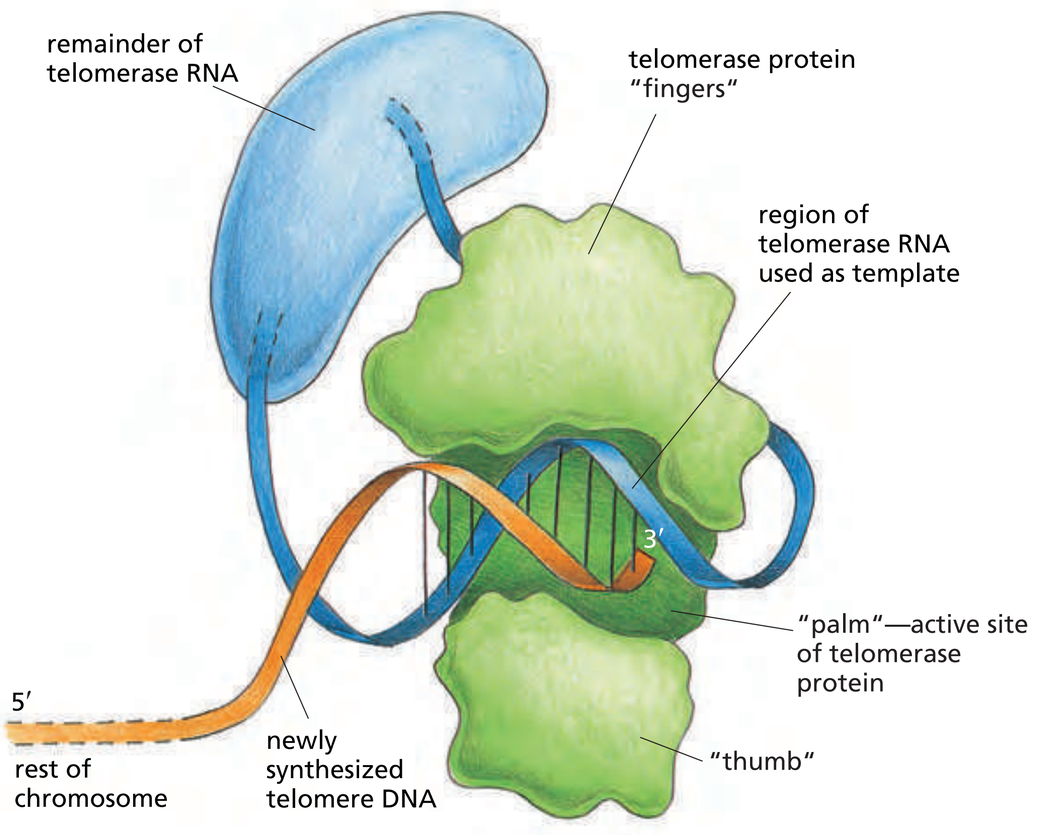
What about nucleosomes?

- **Chromatin-remodelling complexes destabilize** DNA-histones binding to allow the replication fork to move forward
- Broken into a H3-H4 tetramer and H2A or H2B dimers
- New histones (as the total amount has to be doubled) are **synthesised** mostly in **S-phase**
- The assembly is promoted by **histone chaperones**



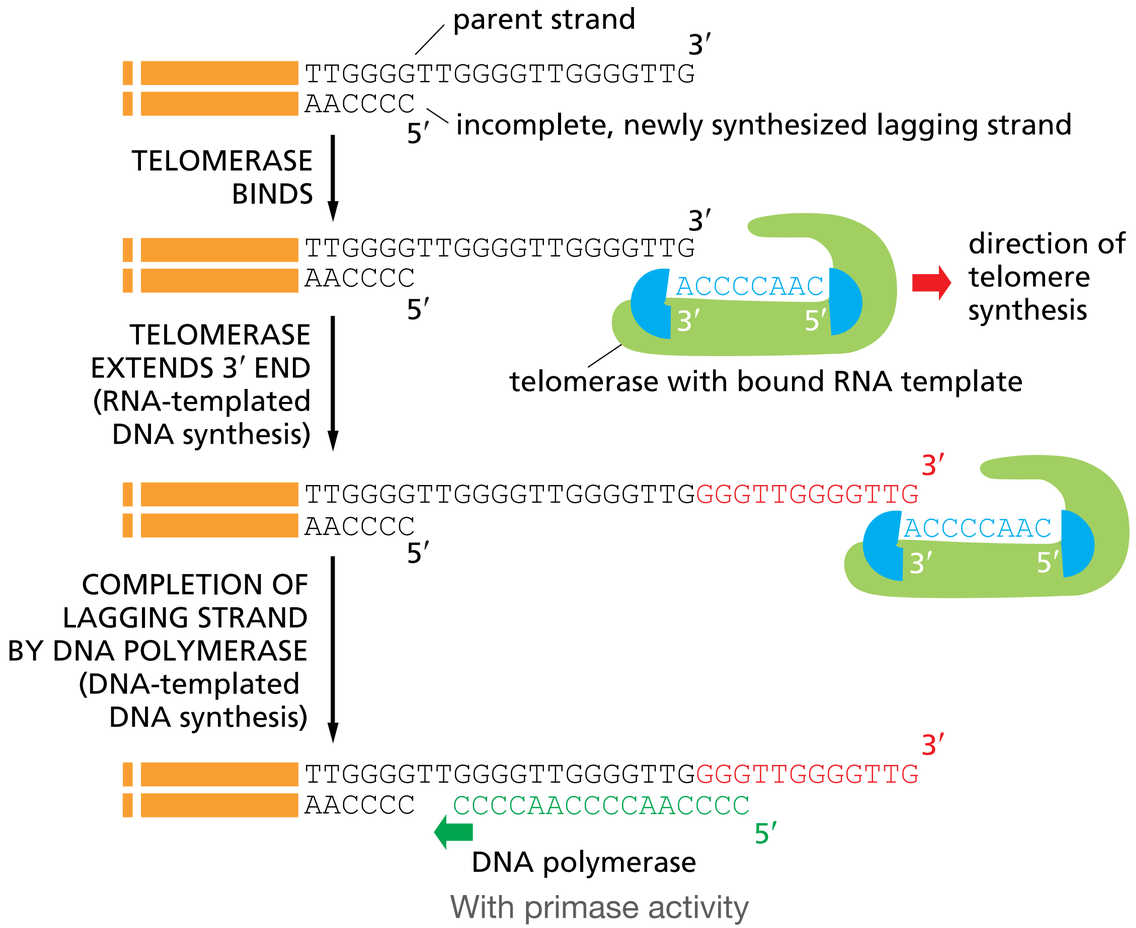
The end of chromosomes

- At the lagging strand, the last RNA primer **cannot be replaced** by DNA (no primer for the DNA polymerase)
- Specific sequences at the end of the chromosomes = **telomeres**
- They contain **repeats** of a short sequence (~ 1000 times)
- In human, the sequence is GGGTTA
- In somatic cells, telomeres get smaller and smaller, which contributes to cell **aging** (death)
- In other cells (germ cells, stem cells, cancer cells,…), this is recognised by a **telomerase** that replenishes this sequence
- It uses an **RNA template** that it part of the telomerase itself



The end of chromosomes

- Similar to **reverse transcriptases** which make DNA using RNA templates
- Then, a **DNA primase/polymerase** finishes strand synthesis



Plan

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- DNA replication mechanisms
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- DNA repair
- Transposition and conservative site-specific recombination

DNA repair

- Tens of thousands **random changes in DNA** per human cell every day; less than 0.02% accumulate as permanent mutations
- The rest is repaired - very **important function**
- Initially studied in **bacteria**
- **Double helix** is ideally suited for repair (one strand remains intact)
- In general,

    1. Damage is **excised**
    2. Original **DNA sequence is restored** by a DNA polymerase
    3. DNA is **sealed** by DNA ligase

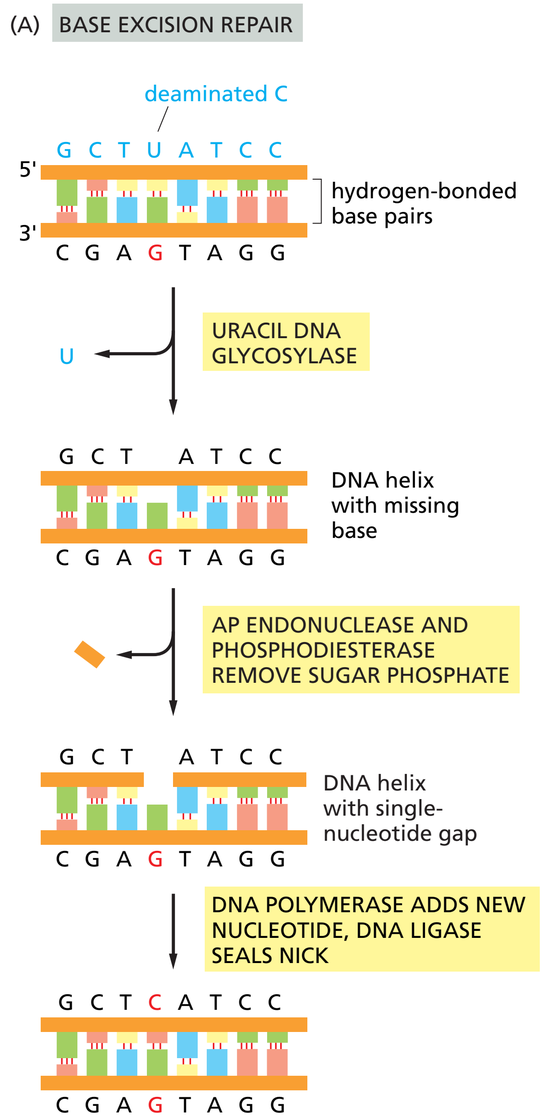
DNA repair

- **Human diseases** associated with decreased repair

TABLE 5–2 Some Inherited Human Syndromes with Defects in DNA Repair		
Name	Phenotype	Enzyme or process affected
MSH2, 3, 6, MLH1, PMS2	Colon cancer	Mismatch repair
Xeroderma pigmentosum (XP) groups A–G	Skin cancer, UV sensitivity, neurological abnormalities	Nucleotide excision repair
Cockayne syndrome	UV sensitivity; developmental abnormalities	Coupling of nucleotide excision repair to transcription
XP variant	UV sensitivity, skin cancer	Translesion synthesis by DNA polymerase ν
Ataxia telangiectasia (AT)	Leukemia, lymphoma, γ-ray sensitivity, genome instability	ATM protein, a protein kinase activated by double-strand breaks
BRCA1	Breast and ovarian cancer	Repair by homologous recombination
BRCA2	Breast, ovarian, and prostate cancer	Repair by homologous recombination
Werner syndrome	Premature aging, cancer at several sites, genome instability	Accessory 3′-exonuclease and DNA helicase used in repair
Bloom syndrome	Cancer at several sites, stunted growth, genome instability	DNA helicase needed for recombination
Fanconi anemia groups A–G	Congenital abnormalities, leukemia, genome instability	DNA interstrand cross-link repair
46 BR patient	Hypersensitivity to DNA-damaging agents, genome instability	DNA ligase I

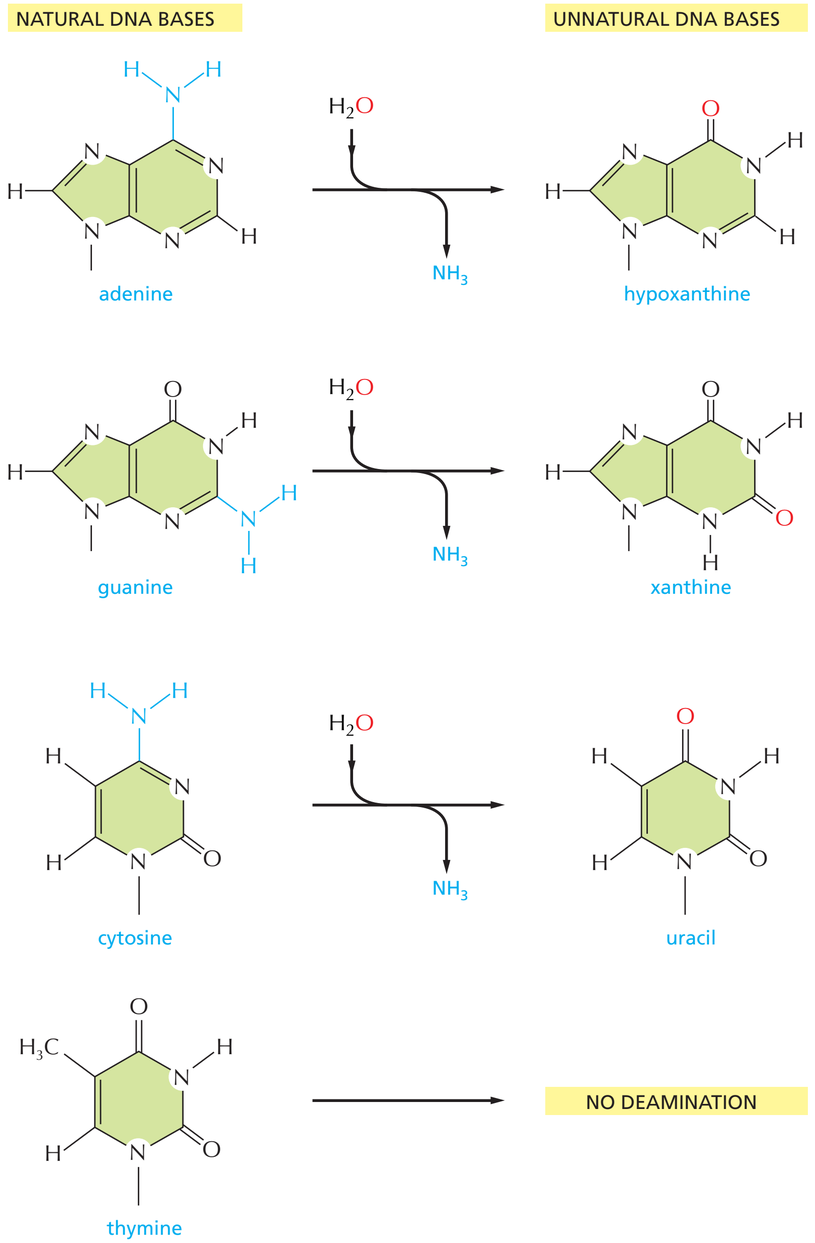
DNA repair mechanisms

- **Base-excision repair:** altered bases are detected and removed



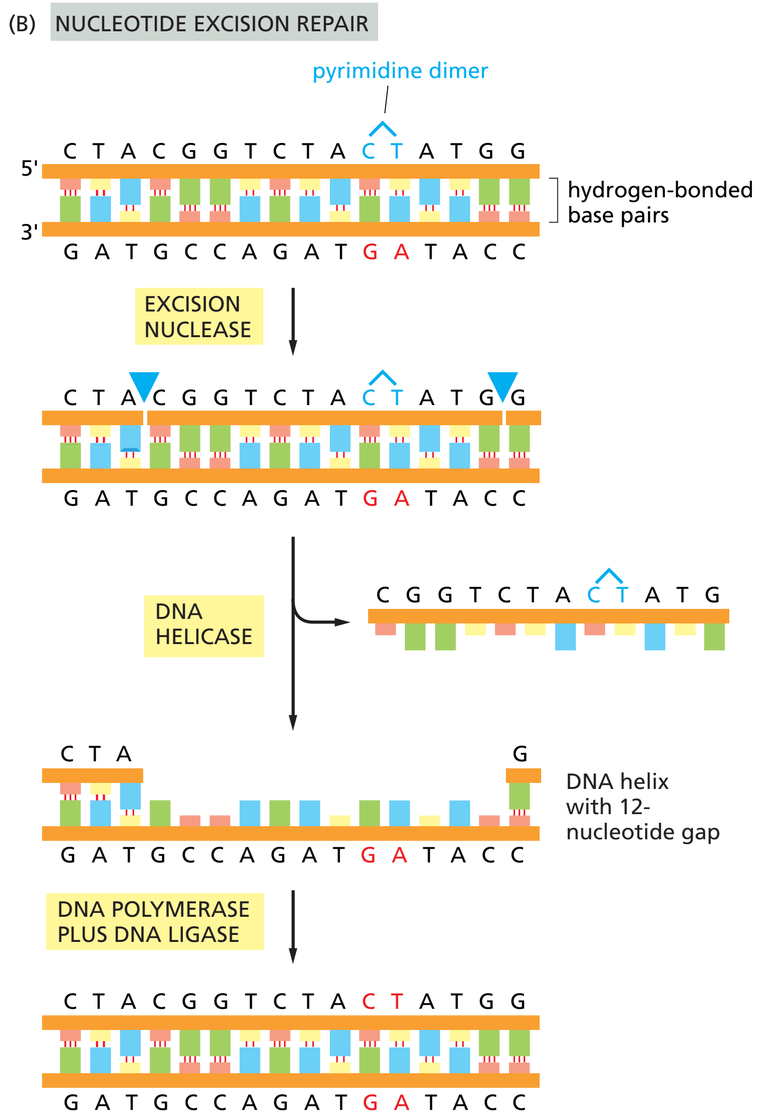
DNA repair mechanisms

- The **chemistry of the bases** facilitates damage detection
- E.g. Every deamination leads to an **unnatural base**



DNA repair mechanisms

- **Nucleotide-excision repair:**
  - Detects **distortions** of the double helix (rather than specific base-change)
  - Cleaves the phosphodiester backbone on both sides
  - A DNA helicase removes the damaged strand
  - DNA polymerase and ligase repair the DNA



DNA repair mechanisms

- How to **direct DNA repair** machineries to **“important” DNA regions**?
  - Coupled with **RNA polymerase** (in charge of transcription)
  - When RNA polymerase stalls at DNA lesions, it **recruits DNA repair**
  - People that lack this coupled action (Cockayne Syndrome) have RNA polymerase permanently stalled on their DNA

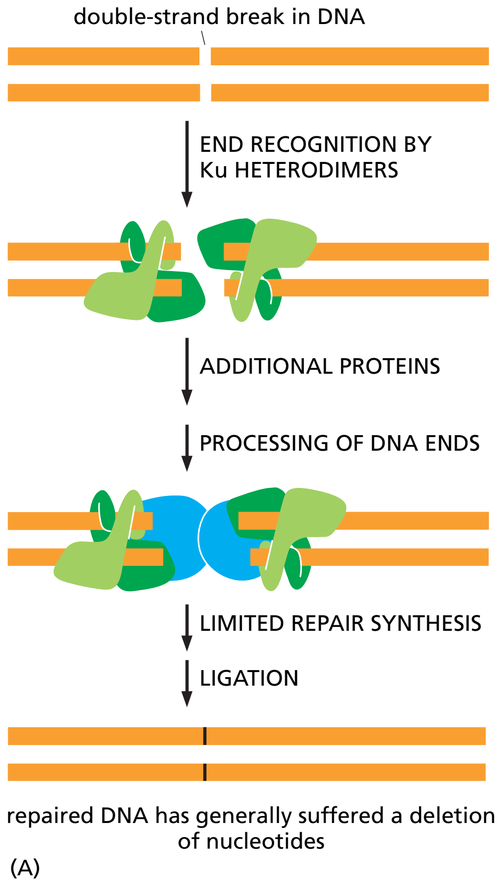
Cockayne syndrome	UV sensitivity; developmental abnormalities	Coupling of nucleotide excision repair to transcription

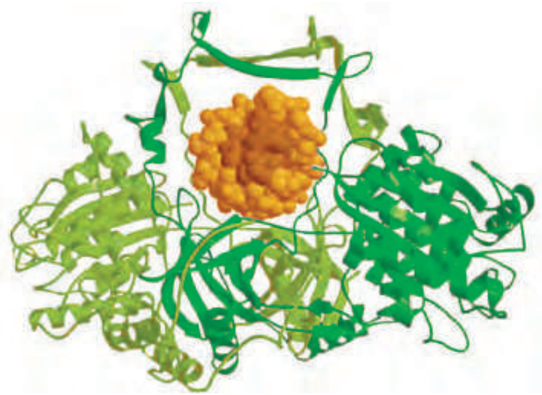
DNA repair mechanisms

- What about **double strand breaks**? No intact template DNA

1. Non-homologous end joining

- Broken ends are brought together
- Ligated
- Lost of a small DNA sequence
- “Quick and dirty”
- Danger: chromosome rearrangement as the system has no way to know if the sequences were together initially
- Telomeres are protected from this





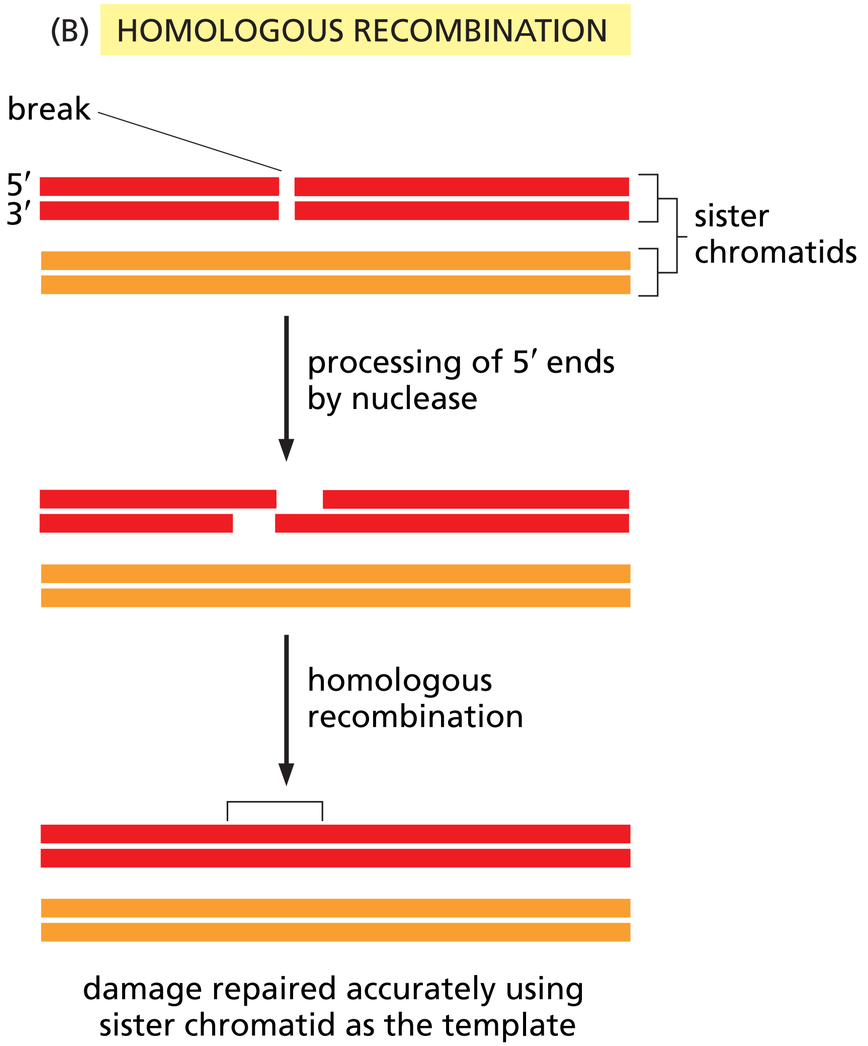
(B)

DNA repair mechanisms

- What about **double strand breaks**? No intact template DNA

2. Homologous recombination

- More accurate
- The sister chromatid is used as a template
- In human, only occurs shortly after DNA replication when sister chromatids are available to serve as template
- More info later

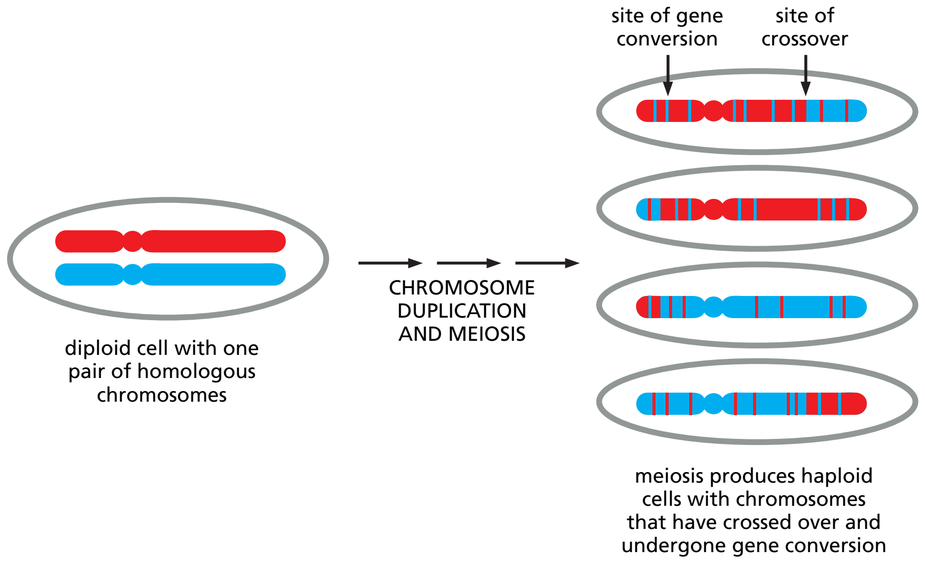


Homologous recombination is not only a repair mechanism!

What is homologous recombination ?

Homologous recombination is the exchange of DNA strands between a pair of homologous duplex DNA sequences

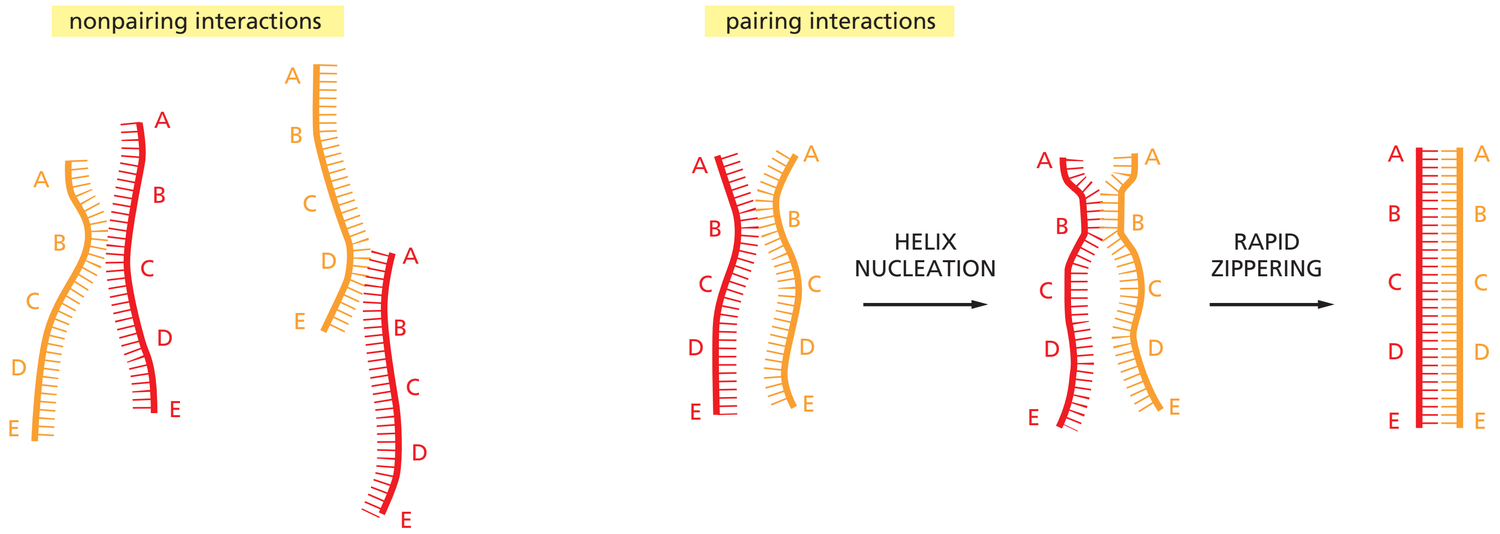
- Can repair many types of **DNA damage** (e.g. double strand breaks)
- **Versatile** mechanism **conserved** in all cells
- During **meiosis,** plays a key role in gamete production (exchange of bits maternal and paternal genetic material) to create new combinations to be passed on to the offspring
- Most of what we know derives from **bacteria** and **viruses**



What is homologous recombination ?

Homologous recombination is the exchange of DNA strands between a pair of homologous duplex DNA sequences

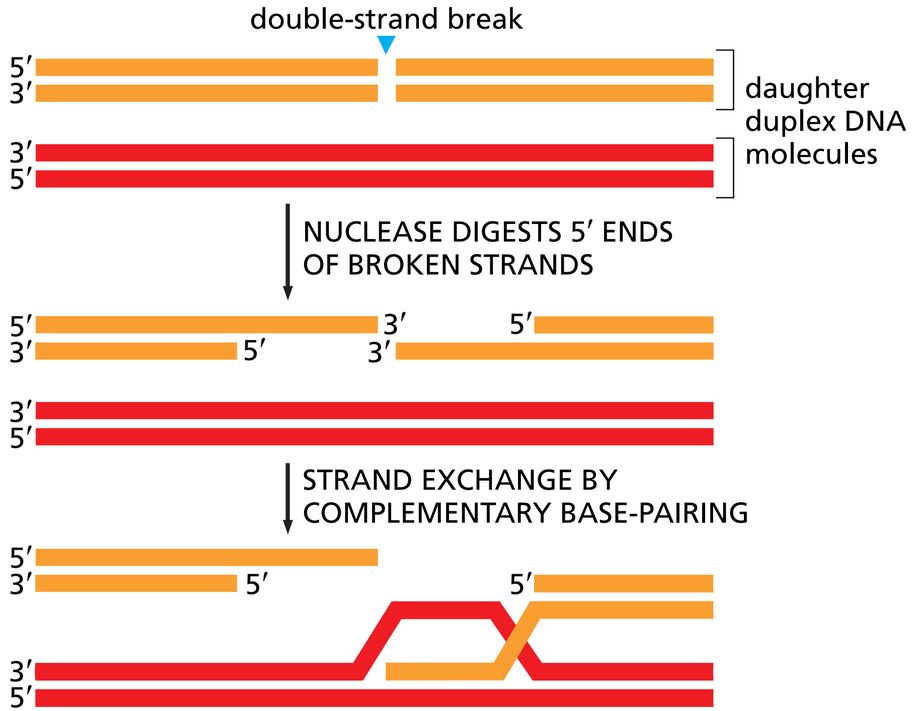
- Only occurs between DNA duplexes that have extensive regions of sequence similarity (homology)
- Concept of **DNA hybridization:**



What is homologous recombination ?

1. The ends of the broken DNA are chewed back by **nucleases** to produce **overhanging single-strand 3' ends**

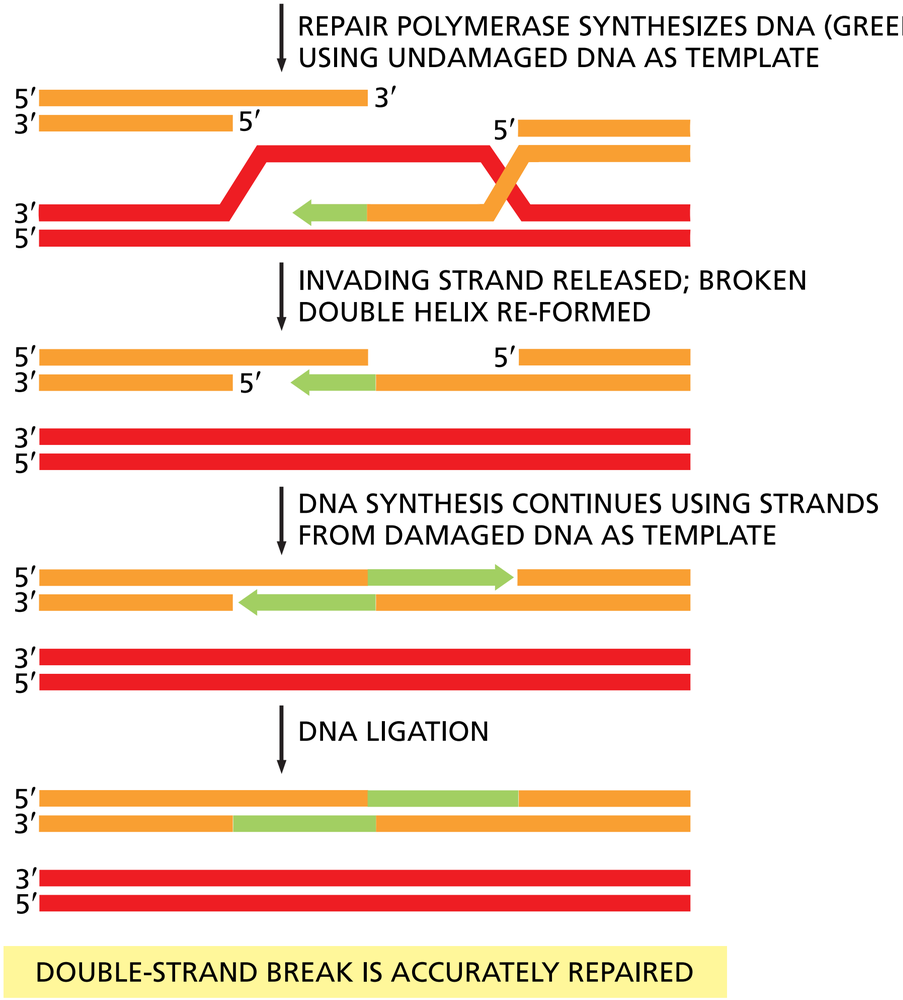
2. **Strand exchange** then happens: the single-strand 3' end searches for a homologous sequence through base-pairing



What is homologous recombination ?

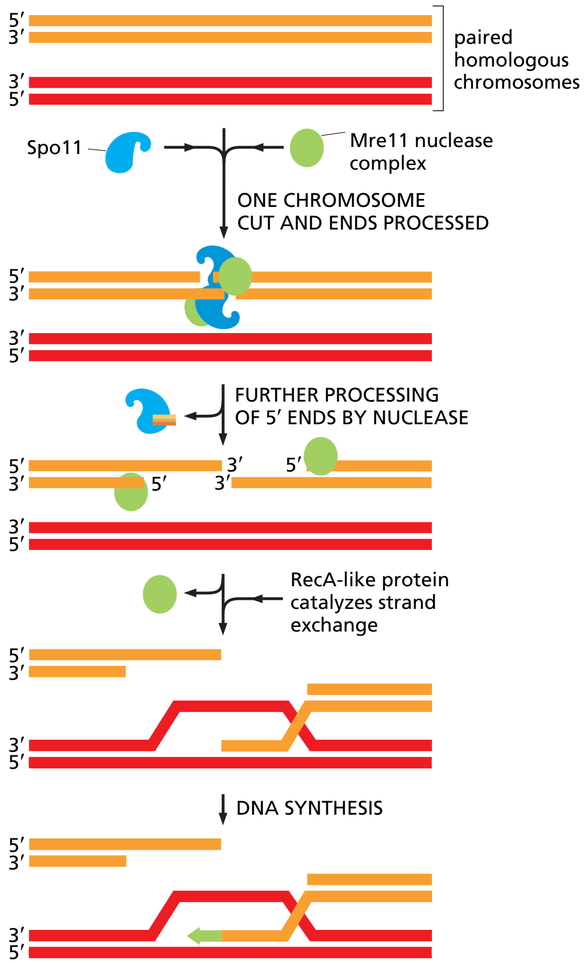
3. **DNA polymerase** extends the invading strand

4. **Ligation** restore the two initial DNA duplexes



What is homologous recombination ?

Homologous recombination during meiosis can generate **chromosome crossovers**



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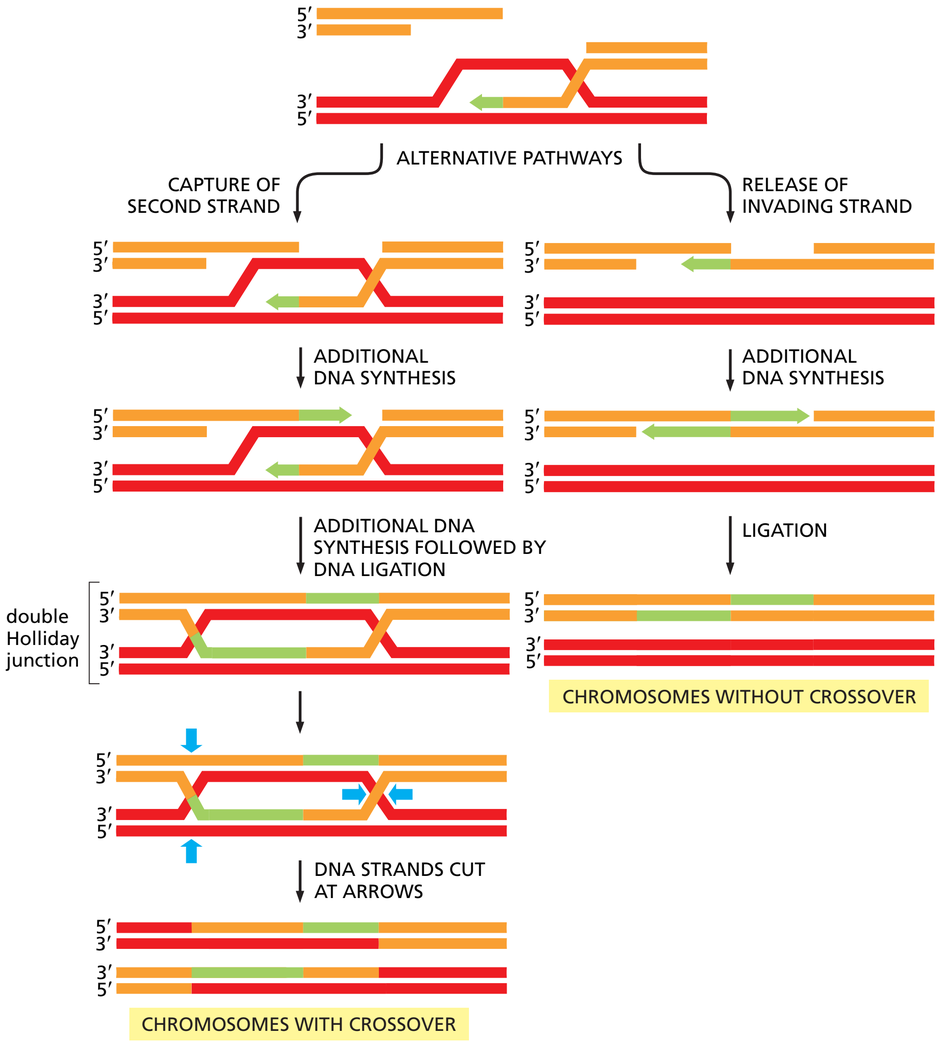

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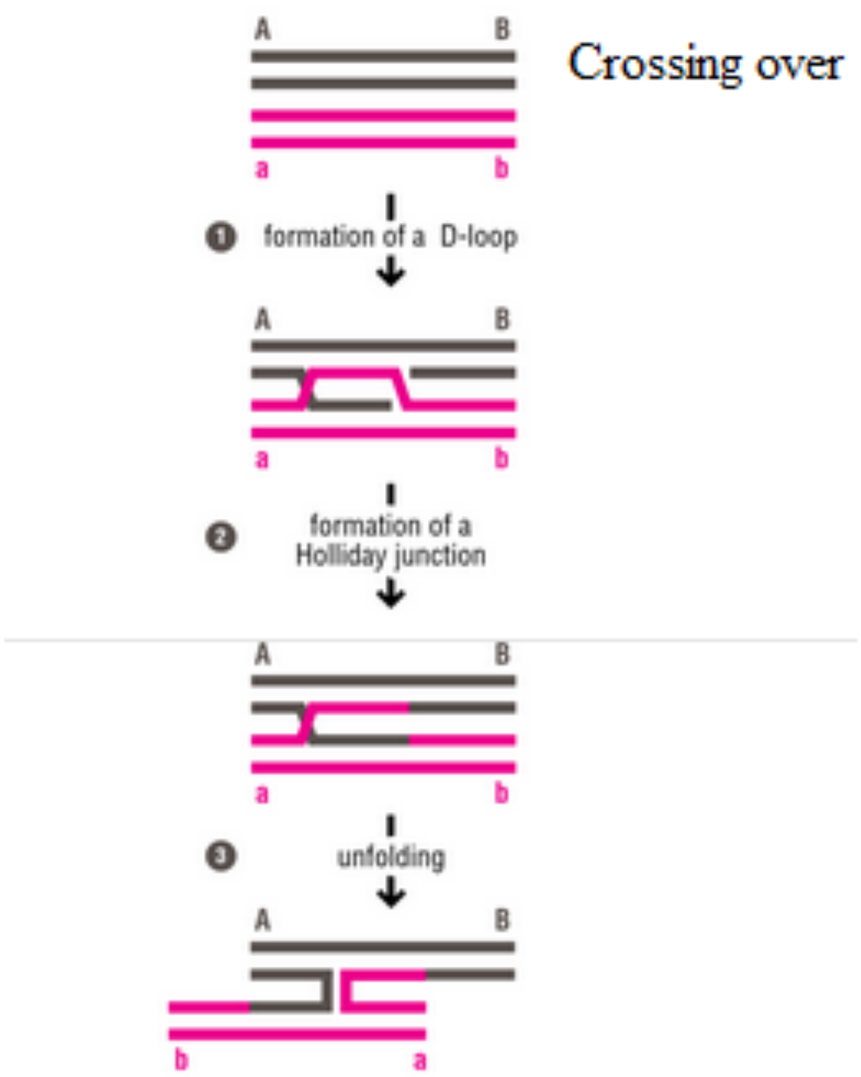
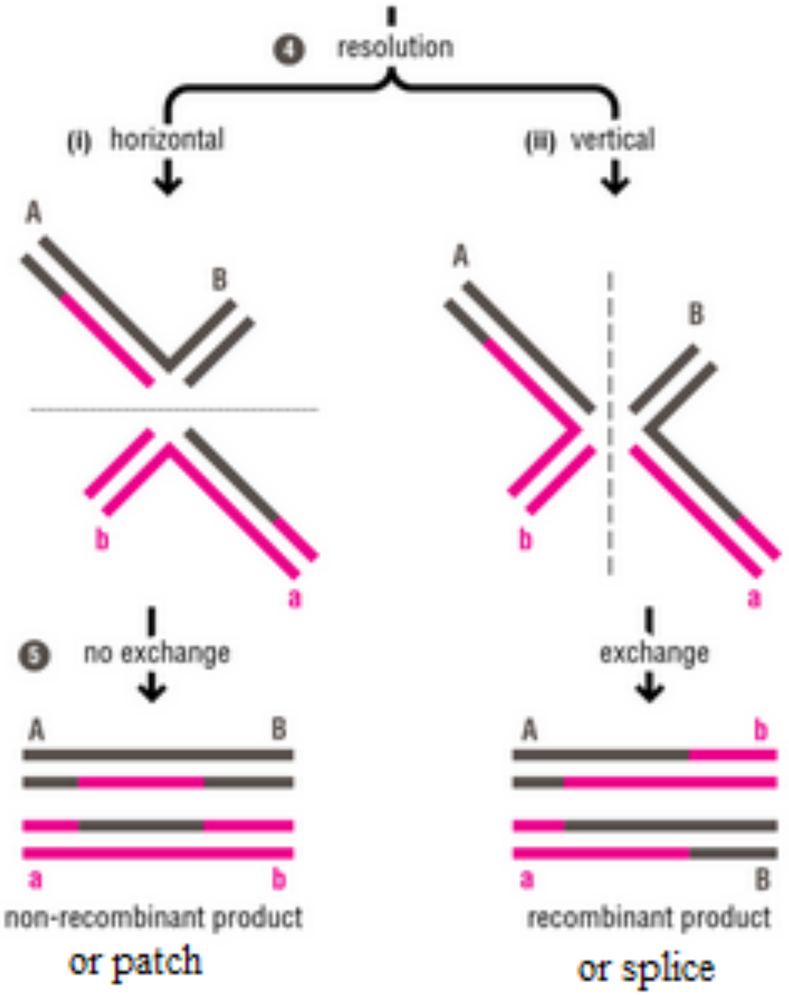




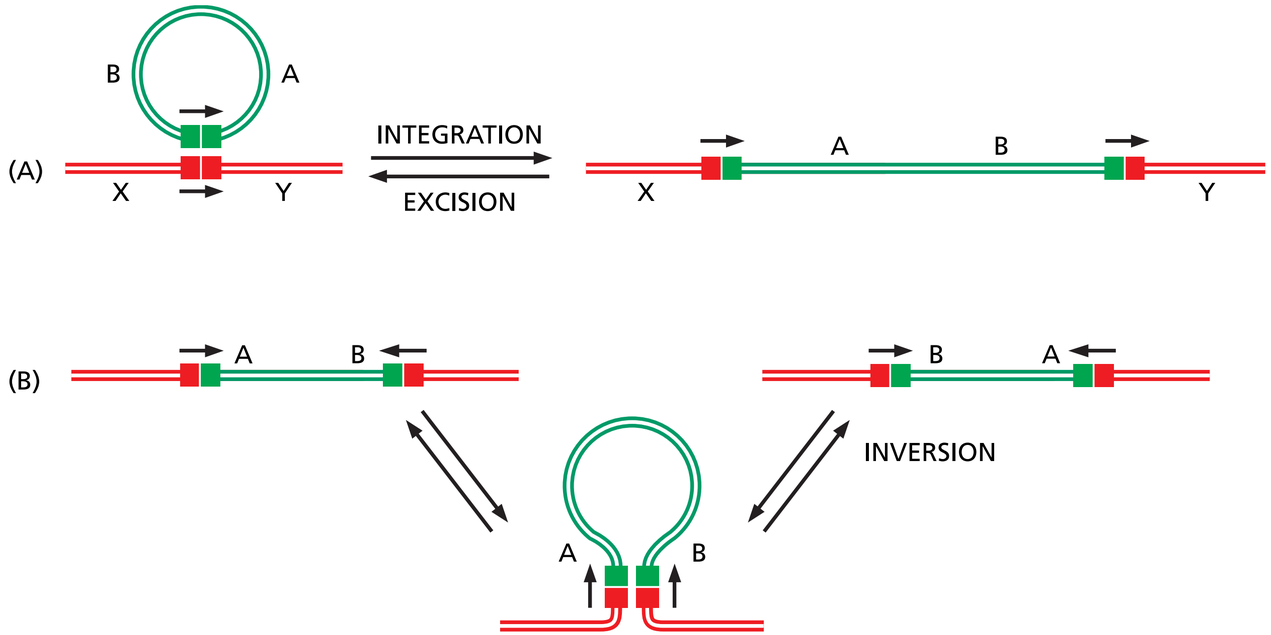
Other examples that use site-specific homologous recombination

1. Insertion of mobile genetic elements/viruses

- For other types of **mobile genetic elements**
- Breakage and joining happens a specific sequences
- DNA integration, excision or inversion can occur
- Also used by some viruses

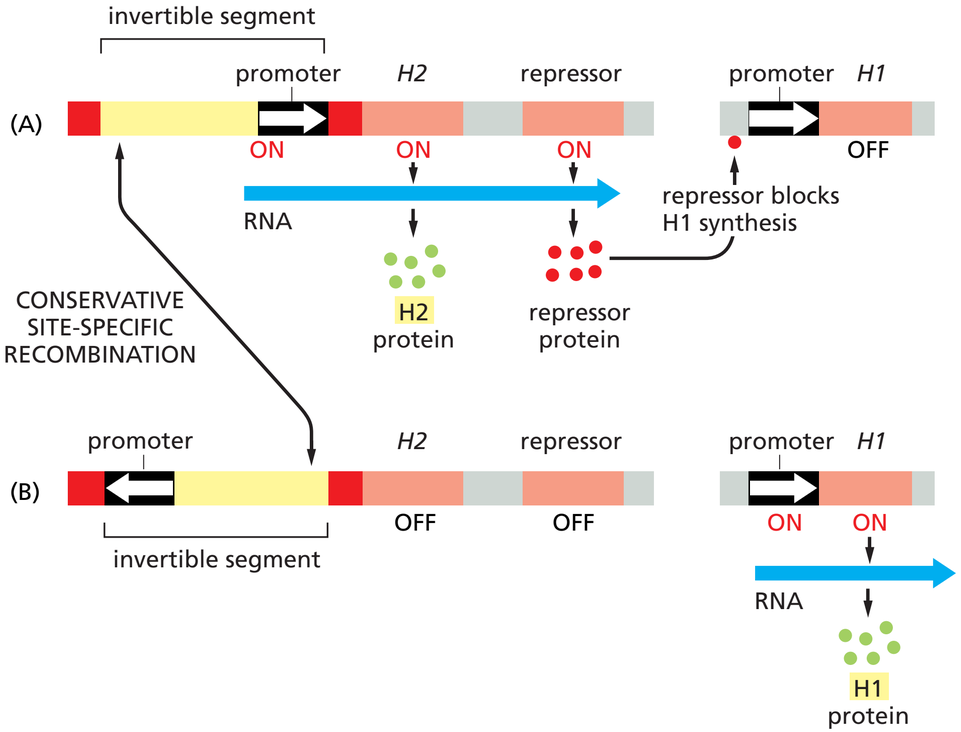
Differences with transposition

- Specific end-sequences on both donor and recipient DNA
- Reaction mechanism are different



2. Phase variation (mostly in bacteria)

- Can be used to switch genes on and off in bacteria (phenomenon called **phase variation**)
- Inversion of piece of DNA that includes the promoter
- Alters the expression of gene (e.g. cell surface flagellin for which bacteria have two genes, H1 and H2)
- Protects bacteria from immune response

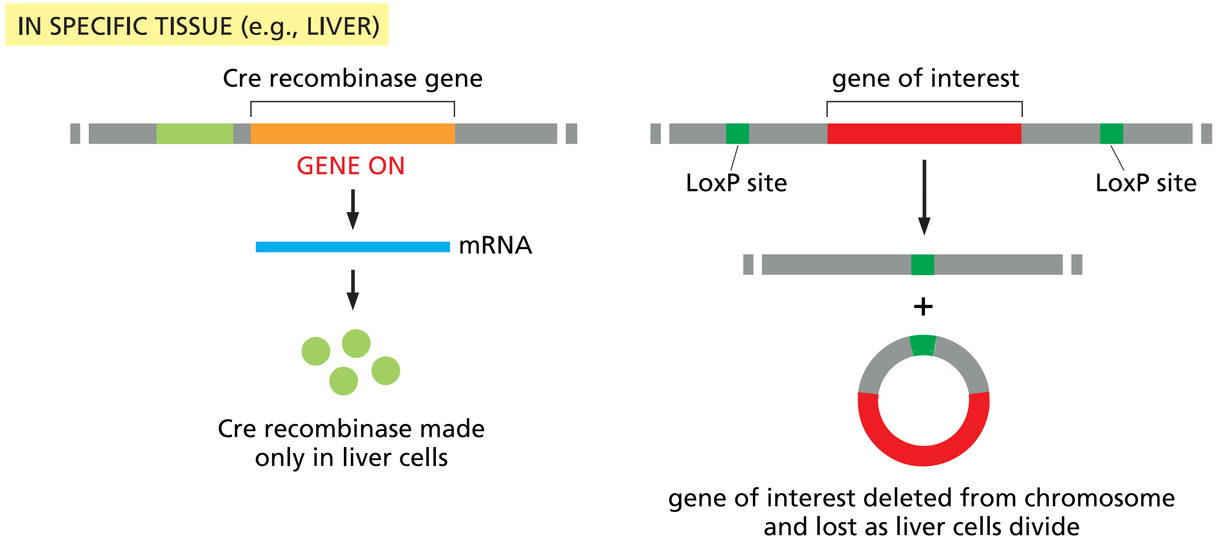


3. Cre-loxP system

- The **Cre-loxP system** is a genetic tool used in molecular biology to control gene expression in a precise, targeted way. It comes from bacteriophage P1, which naturally uses this system to recombine its DNA.

- It is made of
  - ✓ **Cre recombinase** ("causes recombination")
    - An enzyme that recognizes loxP sites in DNA
    - It cuts and rejoins DNA at these sites
  - ✓ **loxP sites**
    - Short DNA sequences (34 base pairs long)
    - The orientation and location of these sites determine the outcome
- How it Works
  - If loxP sites are in the same orientation (→ →): Cre deletes the DNA sequence between them, leaving a single loxP behind
    (Used for conditional knockouts).
  - If loxP sites are in opposite orientations (→ ←): Cre inverts the DNA between them (Used to flip a gene on/off).
  - If loxP sites are on different DNA molecules: Cre can cause recombination and exchange DNA segments

3. Cre-loxP system



- The **Cre-loxP system** is a powerful tool for developmental biologists

Have a nice day!