Topic 18: Intro to DNA and RNA

- Explain chemical structure of nucleic acid polymers without memorizing structures of nitrogenous bases
- Describe beta-DNA and higher order structures formed by RNA, and the important forces that stabilize these structures
- Identify chemical and structural similarities and differences between RNA and DNA

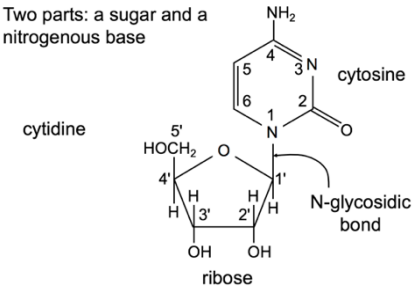
DNA → deoxyribonucleic Acid
- Holds genetic material
- Info passed from parent(s) to offspring
    - Required to produce and organism

RNA → ribonucleic acid
- Template for producing proteins
    - Messenger RNA (mRNA)
- Forming ribosome (along with proteins)
    - Catalyzing protein synthesis
    - Ribosomal RNA (rRNA)
- Carrying amino acids to growing peptide chain during protein synthesis and to ribosomes
    - Transfer RNA (tRNA)
- RNA splicing
    - snRNA
- regulation of gene expression
    - miRNA
- Involved in gene regulation and processes like mRNA splicing and telomere maintanence

DNA → linear polymer composed of deoxyribonucleotide monomers
RNA → linear polymer composed of ribonucleotide monomers
Each **monomer** composed of 3 parts
a) Monosaccharide
b) Nitrogenous base
c) Phosphate

A ribonucleoside

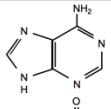
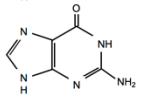
- Two parts: a sugar and a nitrogenous base



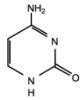
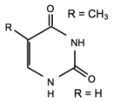
Monosaccharides
- Deoxyribonucleotides contain **deoxyribose**
    - C2' bears **2H atoms**
- Ribonucleotides contain pentose ribose
    - C2' bears one **H atom and one hydroxyl**

Nitrogenous base
- 2 types: purines and pyrimidines
    - both all planar and hydrophobic
- purines → 2
    - found in DNA and RNA
    - adenine (A)
    - guanine (G)
    - join C1' of ribose or deoxyribose via the N9 position
- pyrimidines → 3
    - cytosine (C)
        - found in both DNA and RNA
    - thymine (T)
        - found in DNA but not RNA
    - uracil (U)
        - found in RNA but not DNA
        - identical to T except lacks methyl group (CH3)
    - join C1' of ribose or deoxyribose via N1 position
- anomeric carbon of ribose/deoxyribose → beta configuration when joined to a nitrogenous base

Nitrogenous bases: Purines

Base	Nucleoside	Abbr.	Structure
Adenine	Adenosine	A	
Guanine	Guanosine	G	

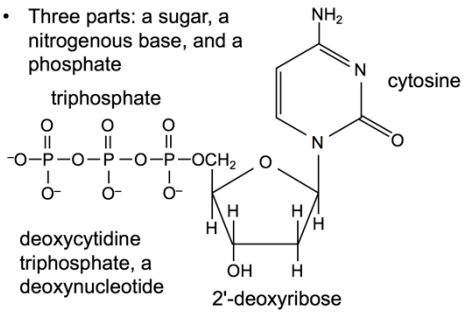
Nitrogenous bases: Pyrimidines

Base	Nucleoside	Abbr.	Structure	Molecule
Cytosine	Cytidine	C		DNA, RNA
Thymine	Thymidine	T		DNA
Uracil	Uridine	U		RNA

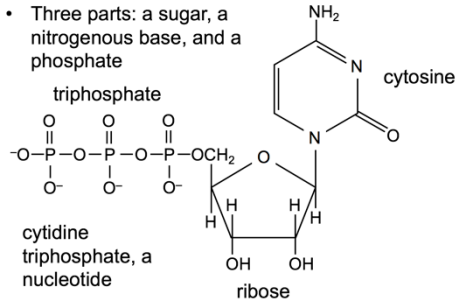
- joined via an N-glycosidic bond
- **nucleoside** → sugar joined to a nitrogenous base
  - 5 common: adenosine, guanidine, cytidine, thymidine, and uridine

Phosphates
- attached to C5' position of ribose/deoxyribose by a phosphoester linkage
- nucleotide formed when phosphate attach to this position of a nucleoside
- there will have all 3 groups
  - sugar
  - base
  - phosphate
- phosphate groups are negatively charged
- names of nucleotides abbreviated
  - using letter for nitrogenous base
  - followed by indication of number of phosphates present
  - i.e. ADP for adenosine diphosphate
  - sometimes, abbreviations for deoxyribose will begin with a small d
    - i.e. dTTP

A deoxyribonucleotide



A ribonucleotide



DNA and RNA structure

Polynucleotides
- sugars joined via phosphodiester linkages between C3' and C5'
- 5' end: 5' carbon not attached to another monosaccharide
- 3' end: 3' carbon not attached to another monosaccharide
- by convention, written 5' to 3'

Mononucleotides are linked together to form a unbranched polynucleotide chain through 3',5' phosphoester bonds
- phosphate group attached to 5' carbon of one pentose forms a phosphoester bond to the hydroxyl group at the 3' position of another pentose
- 5' end
  - end of a polynucleotide at which all the 5' carbon is free
    - not attached to another monosaccharide
- 3' end
  - 3' carbon is free
- sequence of polynucleotide represented by giving letter for each nucleotide base in order
  - i.e. CAAGTG
- polynucleotides written 5' → 3' left to right

single strand of DNA not usually found in cell
- 2 polynucleotide strands tightly but non-covalently associated with each other
  - antiparallel
    - opposite orientation / chemical "polarity"
    - one strand runs from 5' to 3' and other runs 3' to 5'

5' ----------> 3'
3' <---------- 5'

the 2 polynucleotides are wrapped around each other to form a double helix
- double helix normally right-handed
  - if you look at helix along its axis and move gaze along one strand from point close to you to a point further away
    - curve is in a clockwise direction (Fig. 4-14E p. 132)

- base pairs lie inside and phosphates lie outside
    - minimizes charge repulsion between phosphates
    - allows for salt stabilizing of phosphates' negative charges
- 2 types of interactions between the bases stabilize the double helix:

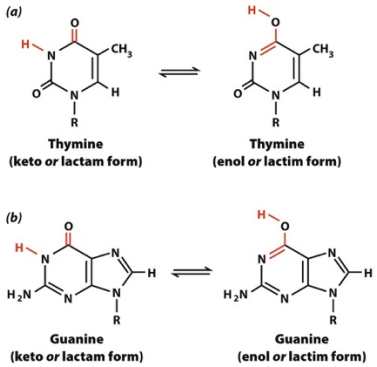
1. **base stacking**
    - bases → planar aromatic rings that are nearly perpendicular to the helical axis
    - flat surface allows them to stack on top of each other
        - stabilized by van der waal interactions
    - atoms in each base pair lie at their optimal van der waal radius from the adjacent base pair
    - not sequence specific
        - any base can stack on top of any other base

2. **base pairing**
    - bases also interact by H-bonds between chemical groups on the edges of their ring structures
    - sequence specific
        - A and T base pair with each other exclusively
        - G and C base pair with each other exclusively
    - H-bond donors and acceptors are complementary to each other
        - AT pair forms 2 H-bonds
        - GC pair forms 3 H-bonds
    - Watson-Crick base pairing
    - Since A only pairs T and G only pairs with C
        - Sequence on one strand dictates sequence on ther
        - If you know sequence of one strand, you know sequence of both
        - Complementary strands

Chargaff's rules
- Base composition of DNA varies among species
- Within a species, base composition is same regardless of tissue, age, or environment
- %A = %T and %G = %C

Diffraction pattern
- pattern suggests double-helix with a periodicity of 3.4 nm containing 10 repeatting units

Correct tautomeric forms of bases
- different tautomers have different hydrogen bonding capabilities



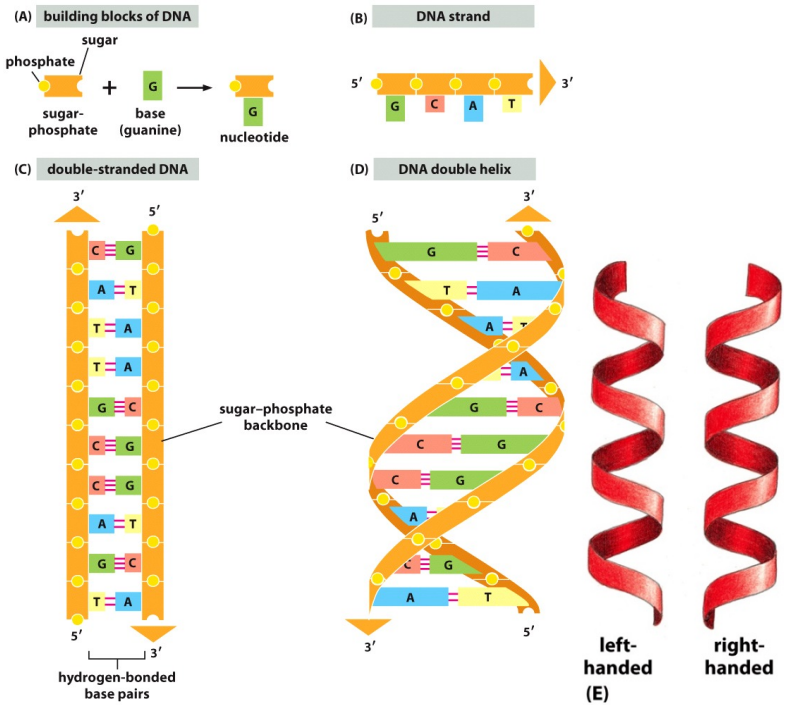
Each base pair consists of one purine and one pyrimidine base
- Gives a uniform base pair size
- The sugar-phosphate backbones stay the same distance apart
- Water is excluded from interior of double helix
    - Cannot compete with bases for H-bonding positions

DNA

- Can form different types of double helices with different geometries
- In cells, DNA usually adopts structure → "B" form
- Features of B-DNA double helix
  1. Narrow, ~2nm (20A) wide
  2. Can be long
     - Human chromosome 1 → 245 million bp
  3. Distance between consecutive bases = 0.34 nm
     - Chromosome 1 → 8.3 cm if stretched out in straight double helix
  4. The 2 grooves between sugar-phosphate backbones are not equal in size
     - Due to positions at which baes are attached to sugar-phosphate backbone and arrangement of H-bonding donors/acceptors
     - Major groove → wider
     - Minor groove → narrower
     - Proteins use the chemical groups that project from the bases into the grooves to recognize the sequence of double-stranded DNA

Double helix: "B-DNA"

- Antiparallel
- Phosphates on outside, bases in middle
- Right-handed

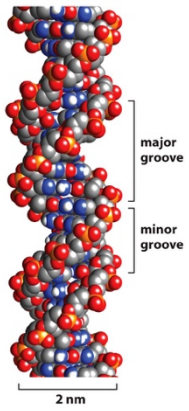


Forces stabilizing the double helix

1. Hydrophobic interactions
   - Planar bases stack on each other
   - Water excluded
   - Not sequence specific
2. Base pairing
   - Hydrogen bonds between nitrogenous bases
   - Water cannot interfere (excluded from helix)
   - Sequence specific

Watson-Crick base pairing

- AT: 2 H-bonds
- GC: 3 H-bonds
- Backbone always same distance apart
- Sequences are complementary



Features of DNA double helix (B-DNA)

- 2nm thick
- distance between base pairs (rise) = 0.34 nm
- one complete turn every 10 base pairs
- can be very long
- major and minor grooves (used by proteins to read sequence)
    - caused by bases not symmetrically oriented to the backbone

RNA → differences between DNA and RNA structures

1. **Sugar in RNA is ribose instead of deoxyribose**
    - Presence of hydroxyl group at the 2' position of ribose makes the phosphodiester bond of RNA sensitive to base (OH-)
    - Unlike DNA, RNA is degraded into mononucleotides in basic solution

2. **RNA contains uracil instead of thymine**
    - Similar bases, differing only by a methyl group (U lacks methyl group)
    - Uracil can form a Watson-Crick base pair with A, just like thymine

3. **RNA usually single-stranded and shorter than RNA**
    - No longer than a few thousand nucleotides
    - RNA backbone folds into unusual shapes that allow for strand to base pair with itself
        - Sometimes a double helix is formed
        - Other structures also arise
    - Base pairing not always strictly according to Watson-Crick scheme
    - Like protein structure, RNA structure is sequence-depended
        - Not true for DNA → any sequence forms the same essential structure

4. **Cells more likely to chemically modify nitrogenous bases in RNA than in DNA**
    - Over 100 different types of modified RNA bases identified
        - Making up 1-2% of bases in tRNA
        - Less than 1% of bases in rRNA
    - Modified bases can influence secondary and tertiary structure of RNA

Summary of differences

- DNA has 2'-deoxyribose; RNA has ribose
    - RNA is degraded in alkaline solution
    - DNA is more stable
- DNA has thymine; RNA has uracil
    - both base-pair with adenine
- in general, DNA strands are longer than RNA strands
- DNA usually double-stranded; RNA usually single0stranded
    - RNA strands base-pair with themselves resulting in irregular structures
- Nitrogenous bases more likely to be modified in RNA than DNA
    - Variety of modifications is larger

Summary

- Nucleic acids are composed of nucleotide monomers
    - Sugar + nitrogenous base + phosphate
- B-DNA is right-handed, antiparallel double helix, stabilized by hydrogen bonding and hydrophobic interactions
- RNA usually single stranded and forms sequence-dependent structures

Topic 19: Chromatin

- Explain different levels of structural organization displayed by DNA in eukaryotic cells
- Describe structure of nucleosome
- Explain effect of DNA packaging on gene expression and identify key mechanism cells use to alter DNA packaging to regulate gene expression

Human DNA must be condensed

- Genome 3 x 10^9 base pairs long
- At 0.34 nm per base pair (102 cm)
- 2 copies of each chromosome per cell
- condensed about 10000-fold to fit inside nucleus

- DNA would be very long if double helices extended in straight line
- Must arrange DNA in compact form so it will fit in nucleus
- DNA must be available for transcription into RNA
- High degree of organization in DNA packing
  - With help of proteins
- Chromatin → complex of DNA and its organizing proteins

DNA packaging is highly organized

- Despite being condensed, DNA must remain available for replication, repair, and gene transcription
- Eukaryotic DNA condensed with help of specialized proteins
- Protein-DNA complex → chromatin

In eukaryotes, DNA is associated with histones (small proteins)

- Not in bacteria
- 5 types: H1, H2A, H2B, H3, and H4
- Fewer than 200 amino acids
- Have many lysine and arginine residues
  - Positively charged
  - Interact with negatively charged phosphate groups of DNA
- Highly conserved

Histones are rich in arginine and lysine

Protein	Arg + Lys
Histone H1	32%
Histone H2A	20%
Histone H2B	22%
Histone H3	22%
Histone H4	25%
Average protein	11%

Histone octamers

- Double helix wraps around histone ocatmer 1.7 times (146 base pairs)
- DNA double-helix wraps around cluster of 8 histone proteins
  - 2 copies each of histone
    - H2A
    - H2B
    - H3
    - H4
- DNA makes 1.7 circuits around histone core, 147 bp apart

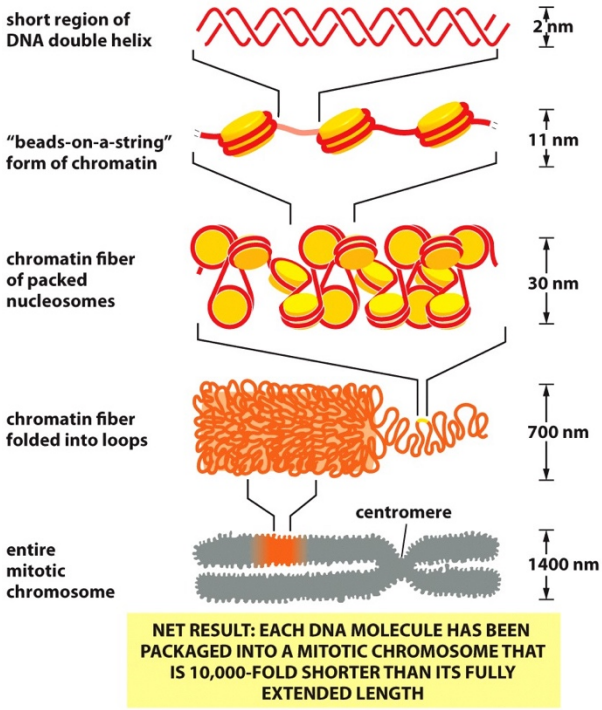
Nucleosomes

- Histone octamers with DNA are called nucleosome core particles
- Core particls are linked by stretches of DNA 3-80 base pairs long
- A nucleosome consists of a core particle and one adjacent linker region
- Nucleosome core particle → wrapped DNA + histone core proteins
  - Linker region (up to 80 bases long) joins the core particles
  - AKA Nucleosome → nucleosome core particle + linker
  - Compact DNA strand by factor of ~3

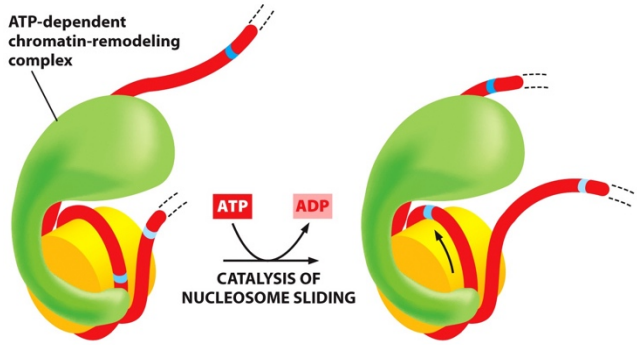
Nucleosomes can pack into a coil → 30 nm chromatin fibre

- 2nd level of DNA packaging
- requires histone H1

- Arrangement of nucleosomes in this fibre is controversial
- Some evidence favors "zig-zag" structure
- Other packaging patterns are possible
- Different arrangements may coexist in cell
- 30 nm fibre stabilized by Histone H1
- length of DNA compacted by factor ~100
- thought to form 30000-20000 bp (30-200kbp) that are anchored to the chromosomal scaffold
    - loops anchored to nuclear matrix, made up of RNA and non-histone proteins
    - scaffold → group of chromatin proteins not part of histone family
    - loops packed together to form chromosome
        - 1400 nm tick in fully condensed form before cell division



- details still discovered about how cell is able to access individual sections of DNA despite all this packing
- chromosome remodeling complexes → use energy from ATP hydrolysis to change arrangement of nucleosomes
    - change nucleosome structure to allow access to DNA for transcription



- chemical modification of histones
    - i.e. phosphorylation, methylation, and acetylation
    - influences affinity of histones for DNA
    - influence stability of higher DNA folding
    - recruit specific proteins to certain regions of DNA

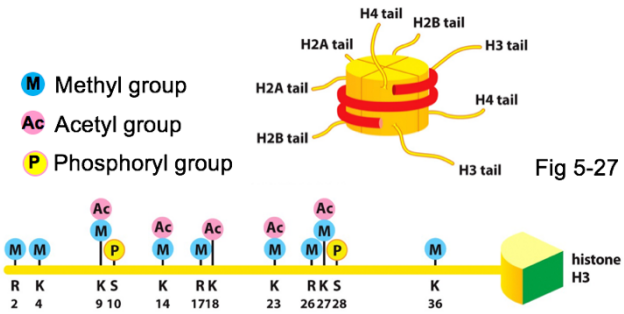


Fig 5-27

Summary

- chromatin is a complex of DNA and protein that results in condensing of DNA
- first level
    - ~200bp nucleosomes composed of histone octamers and linker regions
- second level
    - nucleosomes interact with histone H1 to form 30nm fibres
- third level
    - 30-nm fibres form loops attached to a scaffold
- reorganization necessary to allow access

Topic 20: Replication of DNA

- explain process if replication initiation, including protein and DNA elements involved
- explain process of DNA priming, synthesis, and proofreading, including the protein and nucleic acid elements involved
- explain process of DNA ligation
- draw replication fork and replication bubble, and describe processes occurring at each
- describe arrangement of DNA at telomeres, and explain roles of these structures
- describe how common inhibitors of viral DNA function

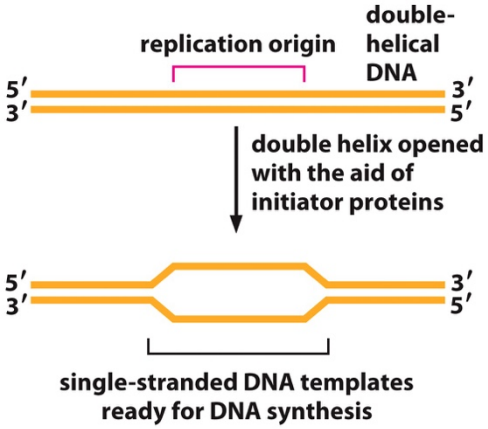
- starting from a single cell, humans must generate many billions of copies of their DNA throughout their lifetime
- DNA must be copied/replicated with great accuracy
- Each DNA strand serves as a template for synthesis of a complementary strand
- DNA polymerase adds new bases according to Watson-Crick base pairing rules

DNA replication

- Each strand of double helix → template for synthesis of new DNA strand
- DNA polymerase makes new strand complementary to template
  - Complex multi-subunit enzymes
- Semi-conservative
  - After replication, each of the 2 daughter double helices contain one original strand and one newly synthesized strand
  - Meselson and Stahl
    - Labeled replicating bacterial cells with 15N
    - Used centrifugation to weigh double and single stranded DNA after various generations
- Steps
  1. Initation
  2. Priming
  3. DNA synthesis (includes proofreading)
  4. ligation

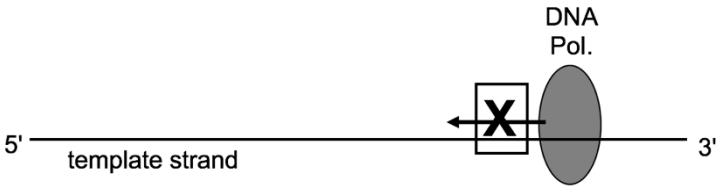
1. <u>Initiation</u>

- Replication starts at specialized DNA sequences → replication origins
  - Prokaryote E coli. (small amount of DNA) → single origin
  - Eukaryotes have more DNA → multiple origins
  - Rich in AT pairs → easier to pull apart than GC base pairs
- Initiator proteins
  - Recognize sequences of replication origins
  - Pull apart 2 strands of DNA at origins
- After DNA pulled apart, helicase and other proteins can bind to the DNA
- Helicase
  - Binds to single-stranded DNA
  - unwinds double helix while consuming ATP
- many copies of Single-strand binding protein bind to unwound DNA
  - Prevents unwound regions from reforming base pairs (re-annealing)

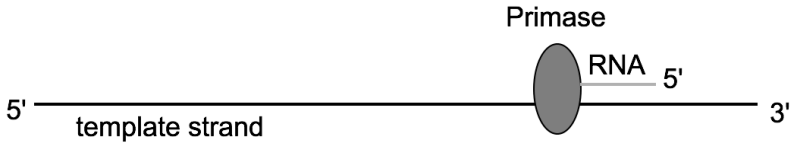


2. <u>Priming</u>

- DNA polymerase can't synthesize DNA strand using only template strand
  - Needs something to attach nucleotides



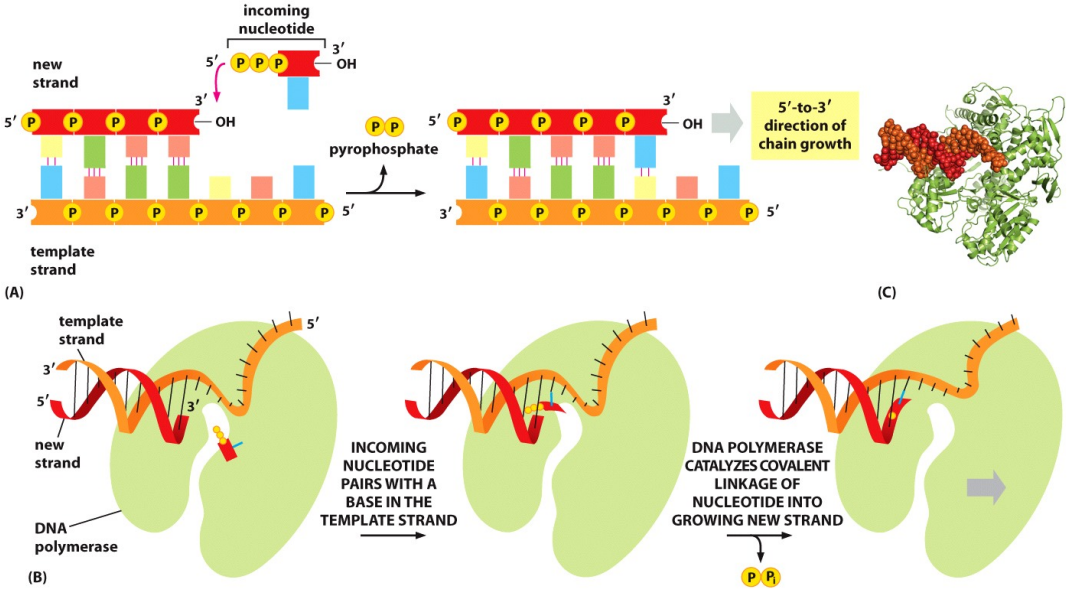
- DNA polymerase needs a primer to synthesize DNA
- Primase (enzyme) synthesizes short (~10-20 nucleotides long) RNA primers on separated DNA strands
    - 5' to 3' direction



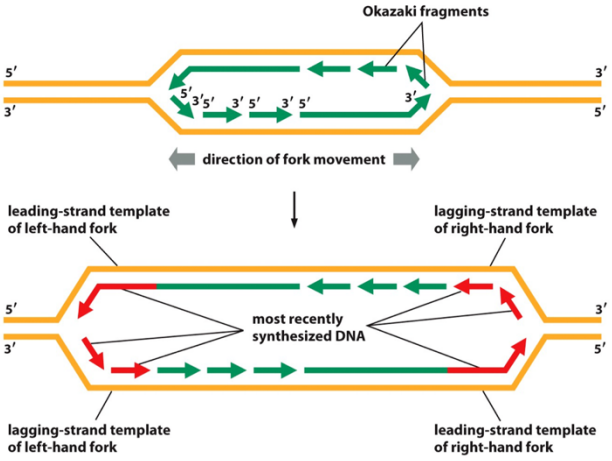
3. DNA synthesis

- DNA polymerase synthesizes in 5' to 3' direction
    - Extends primer from its 3' end

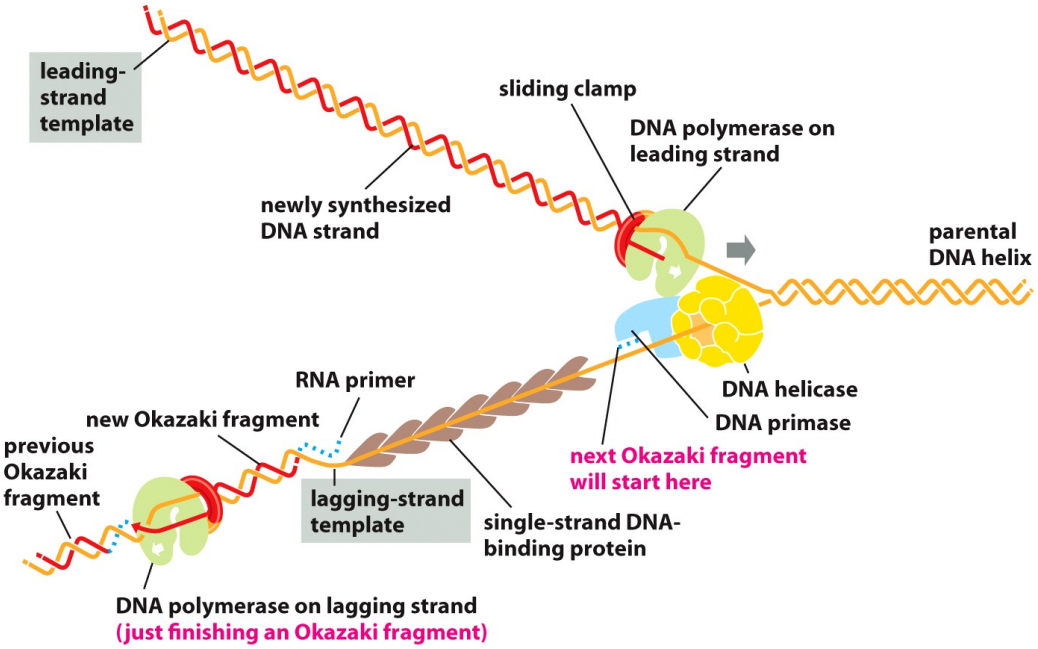
- At each position of single-stranded template DNA
    - DNA polymerase adds complementary base from deoxyribonucleoside triphosphate (dNTPS) in the surrounding solution



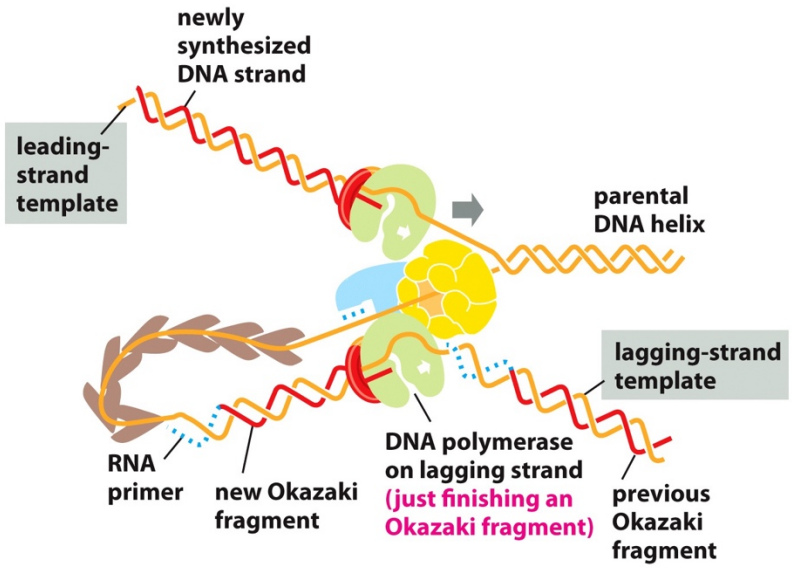
- New DNA always added to the 3' end
    - Numerous DNA molecules 100-200 nt long (in humans) on lagging strand are called Okazaki fragments
- Mononucleotides incorporated into new DNA strand
- Pyrophosphate is the by-product
- Synthesis occurs in both directions from the replication origin
    - At the replication forks
    - Helicase travels at front of fork, unwinding the DNA
    - DNA polymerase follows, making DNA
    - Sliding clamp protein keeps DNA polyermase on template
    - Leading strand: continuous synthesis (~5x10^5 nt per primer)
- 2 DNA polymerase complexes associate with helicase



    - each synthesize DNA using a different template strand
- sliding clamp protein
    - helps DNA polymerase stay associated with template strand
    - forms a ring-shaped structure around the DNA
- double stranded DNA → antiparallel
    - replication on one of template strands proceeds naturally
        - DNA polymerase travels with replication fork in the 3' to 5' direction synthesizing complementary DNA in the 5' to 3' direction
        - This template strand = leading strand
            - Synthesis is continuous
            - DNA synthesis can continue for long distances using 1 primer (5×10^5 nucleotides)
- Complementary strand → lagging strand
    - DNA synthesis must occur in the opposite direction to replication fork movement
    - discontinuous synthesis to form okazaki fragments (connected later by DNA ligase)
        - in E. coli, okazaki fragments 1000-3000 nucleotides long
            - only $1/10^{th}$ this length in humans
        - needs primers for synthesis on leading strand
- replication on leading strands and lagging strands occurs at the same time



- 2 DNA polymerase associate with each helicase



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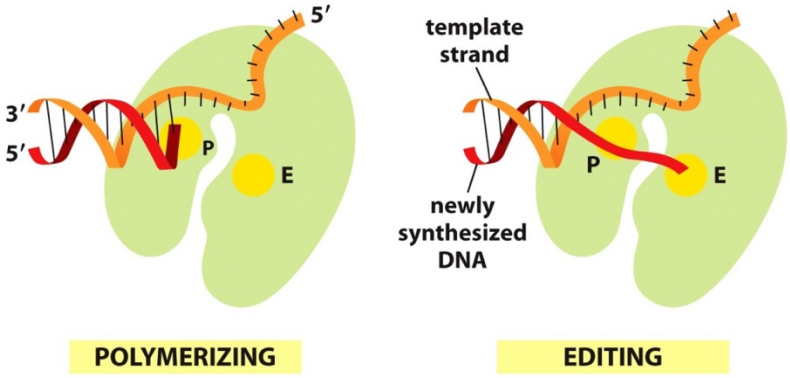
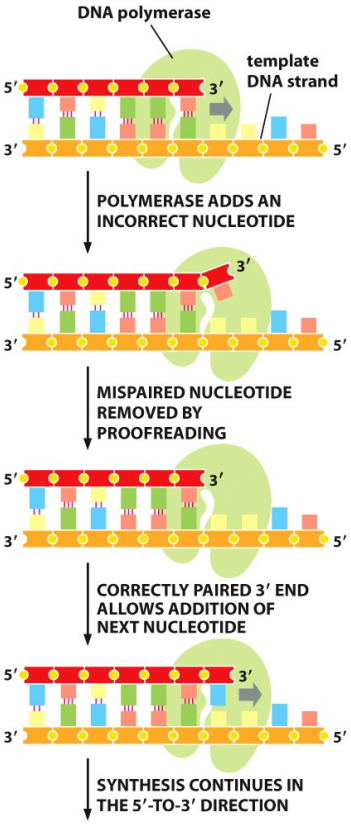
proofreading

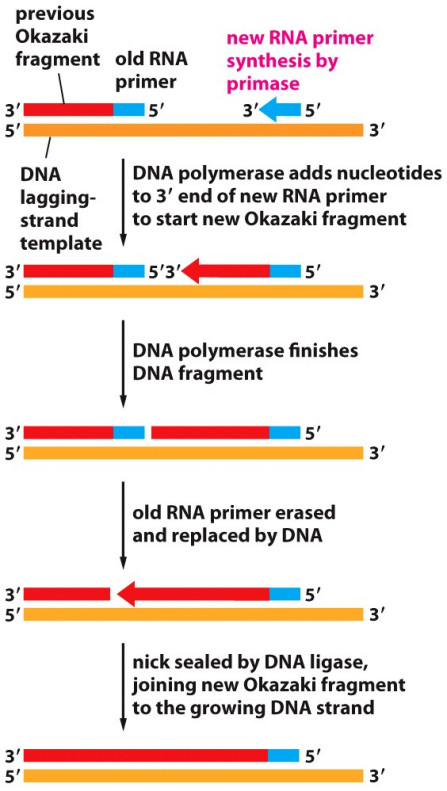
- DNA polymerase can add wrong nucleotides or an extra nucleotide, or can sip a nucleotide
- Error rate of DNA polymerase in Humans: 1 in 10^5 bases
- 3' to 5' exonuclease activity removes errors and synthesis continues
- errors can take form in
  - substitution → adding incorrect base
  - insertion → adding extra base
  - deletion → not adding enough base
- in addition to polymerase activity, DNA polymerase has 3' to 5' exonulcase activity in a distinct active site
  - remove mononucleotides from 3' end of DNA srand
  - proofread newly synthesized DNA strand
  - remove any erroneously incorporated nucleotides
- after incorrect nucleotides have been removed, synthesis resumes from point of error
- proofreading increase accuracy of DNA replication 100-fold
  - 1 error in every 10^7 bases



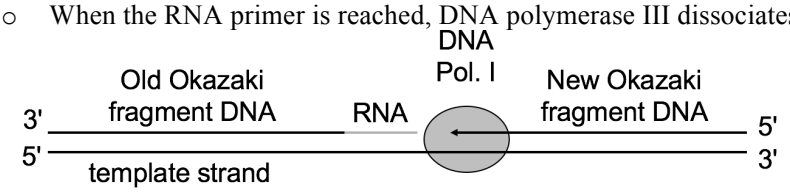


4. <u>Ligation</u>

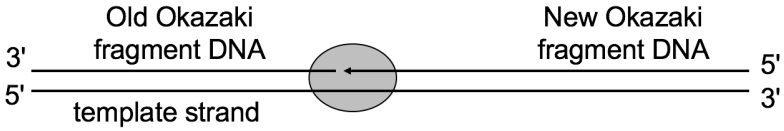
- DNA synthesis continues until the polymerase meets the RNA primer of a previously synthesized DNA fragment
- When DNA polymerase completes okazaki fragment, it encounters RNA primer from the preciously synthesized fragment
- In bacteria, the replicated DNA polymerase dissociates from template and repair DNA polymerase binds
- Besides synthesizing DNA, repair polymerase has 5' to 3' exonuclease activity → degrade RNA primer
- "nick translation"
  - results from repair polymerase degrade primer and synthesize DNA at the same time
- when all RNA has been replaced, repair polymerase dissociates from double helix
  - nick in newly synthesized DNA sealed by DNA ligase
- bacterial DNA ligase requires NAD+
- DNA ligase of other organisms hydrolyzes ATP to AMP and PPi
- Ligase requires substrate DNA strands to have 3' hydroxyl and 5' phosphate
- At some point, the polymerase runs to a previously synthesized frame
- There are 2 replication origins on the same chromosome
- When polymerase meets the previous strand
- It can only incorporate one nucleic acid at a time
- Does not take 2 existing strands and join them together
- So we need DNA ligase to join these 2 strands together



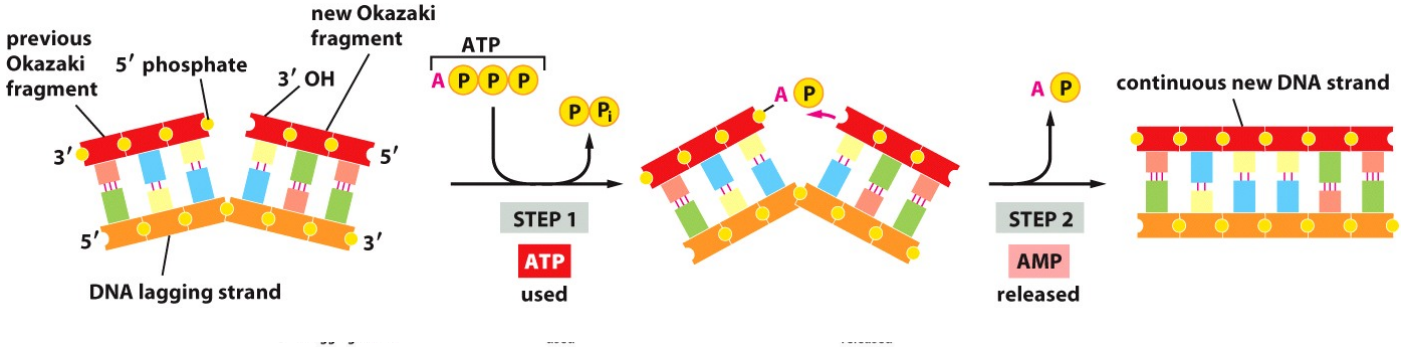
- Ligation in bacteria
  - When the RNA primer is reached, DNA polymerase III dissociates and DNA polymerase I binds

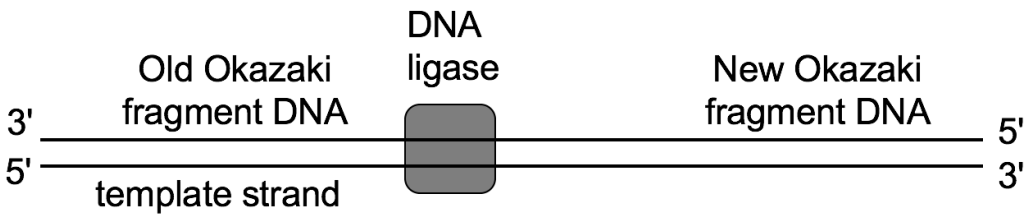


  - DNA polymerase I degrades the RNA primer with its 5' to 3' exonuclease activity, and synthesizes replacement DNA



  - DNA ligase joins the 2 DNA strands
  - Requires a free 3' – OH and a 5' phosphate

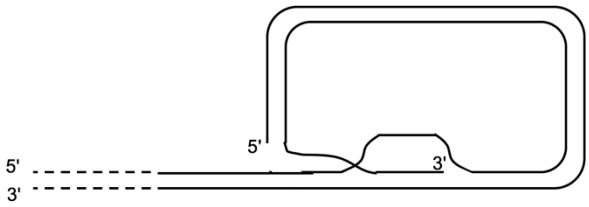




  - Result: an intact DNA double helix

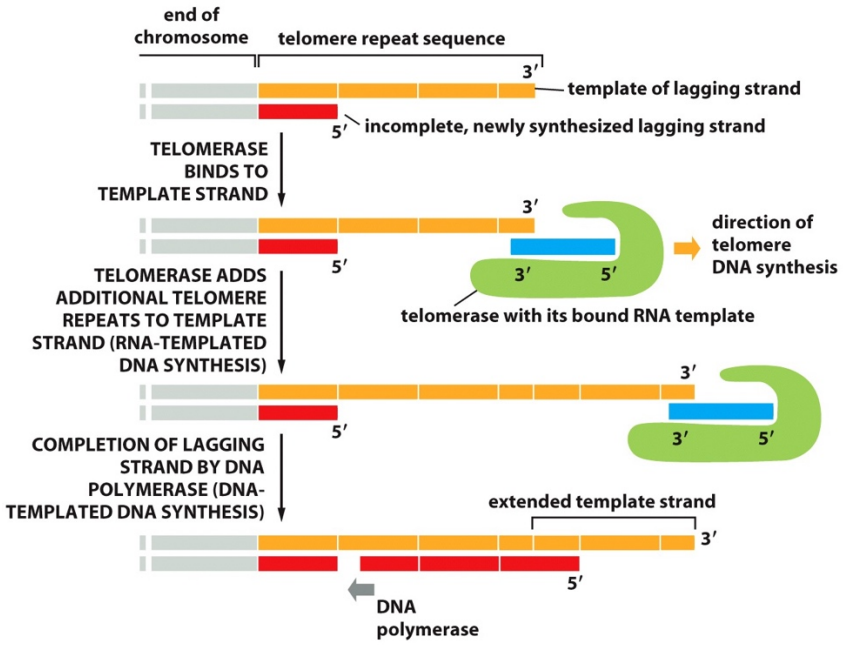
Telomeres

- Protein-DNA structures formed at ends of chromosomes
  - 5000 to 15000 bp double-stranded region composed of many short nt repeats (TTAGGG in humans)
  - 30-200 nt 3' overhang loops back and displays earlier section of same strand
  - many specific proteins associated with this DNA structure
- telomeres enable cell to distinguish between end of chromosome and a double-strand break in the middle of the chromosome
- telomeres help with replicating lagging strand at ends of chromosome



  - cell unable to synthesize DNA complementary to the very end of the lagging strand because RNA primer is needed
  - chromosomes shorten with each cell generation
    - lost DNA does not contain genes
  - therefore, chromosomes get shorter each time DNA is replicated
- telomeres provide "extra" DNA at ends of chromosomes so coding DNA is not lost during replication
- chromosome shortening → part of biological clock that determines number of generations at which cells stop dividing
  - may contribute to aging process

- telomerase
  - enzyme
  - special DNA polymerase
  - synthesizes and lengthens telomeres
  - in somatic cells, telomerase expression stops shortly after birth
    - expressed during embryo development and germ-line cells
  - abnormal expression contributes to making cancer cells immortal
- replication problem
  - Will start eating into the coding sequences
  - To prevent that
  - Once that the chromosomes get short enough
  - Cell will get into phase of synthesis → gets quiet and doesn't't reproduced anymore
  - Repeat until its long enough
  - So you'll get the synthesis of another okazaki fragment
  - So your chromosome can get longer



<u>some antivirals target viral DNA synthesis</u>

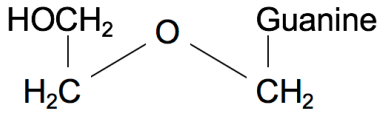
- when these drugs are incorporated into DNA, <u>synthesis stops because there is no free 3' OH</u>

antiviral nucleoside analogues Azido thymindine (AZT)

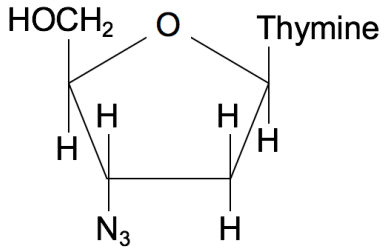
- Aka zidovudine/Retrovir
- Antiviral agent used to combat human immunodeficiency virus (HIV)
- HIV → retrovirus
  - Small single-stranded RNA virus
  - Once in cell, virus
  - RNA transcribed to DNA through reverse transcription using enzyme reverse transcriptase
- AZT taken orally and converted to triphosphate form by patient's cells
  - Can be used as substrate in place of TTP during DNA synthesis
  - Incorporated into newly synthesized DNA strand
  - Has azide (N3) instead of hydroxyl group at 3' carbon
    - Next nucleotide cannot be added to strand
    - DNA synthesis stops
    - Prevents formation of new virus particles
- AZT triphosphate inhibits reverse transcriptase 100 times more effectively than it inhibits human DNA polymerase
  - Due to viral enzyme having higher affinity for AZT than for dTTP

Acyclovir

- Selective inhibiter of herpes virus DNA polymerase
- Causes chain termination when incorporated into growing DNA strand



acyclovir
anti-herpes



zidovudine (AZT)
anti-HIV

Summary

- Old DNA strands used as templates for new DNA synthesis (semi-conservative)
- Steps
    - Initiation
    - Priming
    - DNA synthesis (and proofread)
    - Ligation
- Linear chromosomes end in telomeres that protect coding sequences

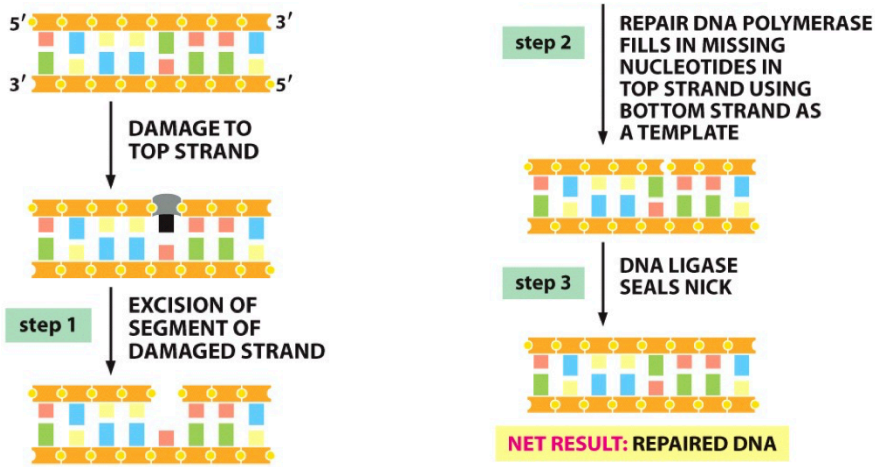
Topic 21: DNA repair

- Describe and differentiate between different kinds of commonly observed DNA damage
- Predict which repair mechanism will be used to repair a particular instance of DNA damage
- Predict consequence of DNA damage that is not repaired before next round of replication
- Describe role of translesion DNA polymerases as they relate to DNA damage

DNA damage

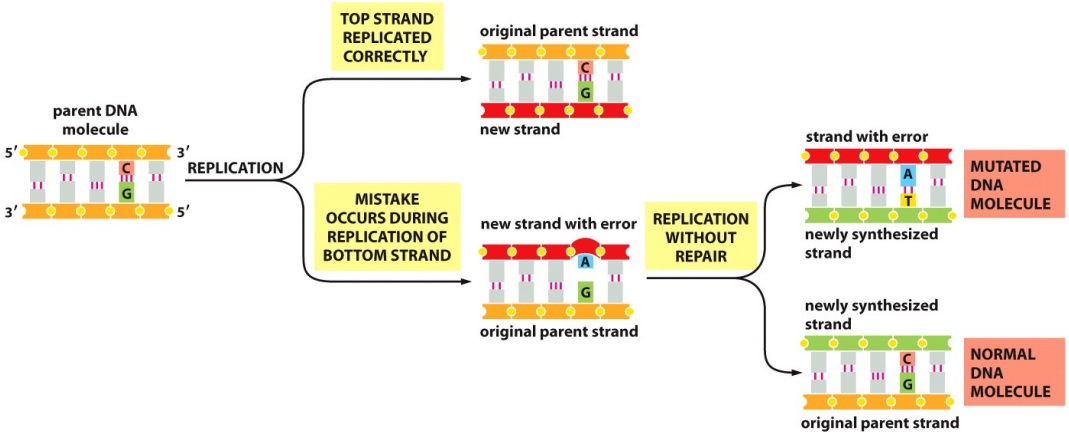
- To preserve genetic info, cell must guard against unintended changes in sequence/structure of DNA
- Any unintended physical or chemical change in DNA or its sequence
- DNA molecules are huge and are prone to damage
- To try to preserve nucleotide sequence, cells have developed mechanisms for repairing DNA damage

Basic mechanism of DNA repair



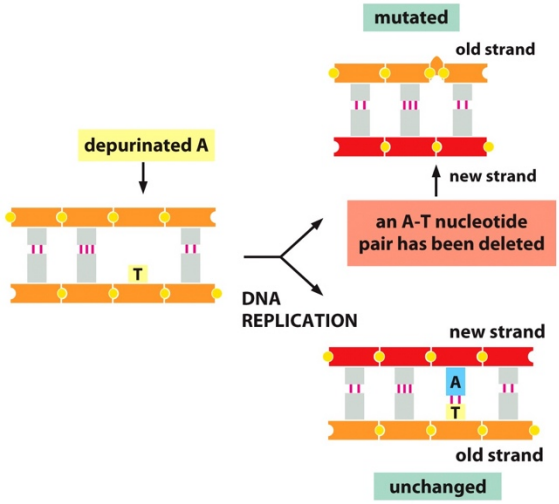
<u>Causes of DNA damage</u>

1. **Copying mistakes**
   - DNA polymerase makes error about once every 10^7 nucleotides
   - Possible outcomes
     - Mismatched base pair
       - Lead to change in DNA sequence or mutation in one of daughter double helices after DNA replication
     - Insertion or deletion of one or more bases



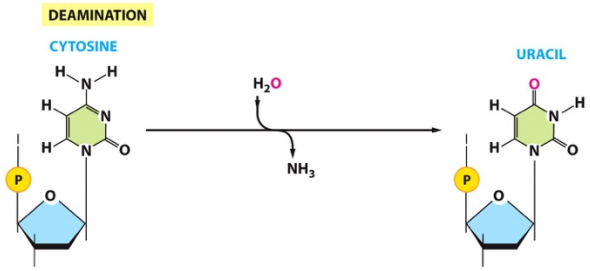
2. **Depurination**
   - Acids promote loss of entire guanine or adenine base, resulting in an abasic site
     - Only lose the purine base itself
   - Sugar phosphate backbone remains intact

- Abasic sites block resplication by normal replicative DNA polymerase
    - When this DNA polymerase stalls, one of several translesion DNA polymerases recuited to site
- Translesion DNA polymerase
    - Able to synthesize DNA past site of damage
    - But because template has no base, they are likely to either
        - Skip that position or
        - Introduce mutation in newly synthesized strand
- After translesion polymerase has synthesized DNA past site of damage, normal replicative DNA polymerase resumes DNA synthesis
- Result
    - Blocks DNA replication
        - can be overcome by error-prone translesion DNA polyermases
    - can (mis)incorporate base opposite abasic site



3. Deamination

- Spontaneous conversion of amine group to carbonyl
- Most commonly happens to cytosine
- May lead to mutation
- Result
    - Substitution of U for C
        - C converted to U
        - Can leave the G alone



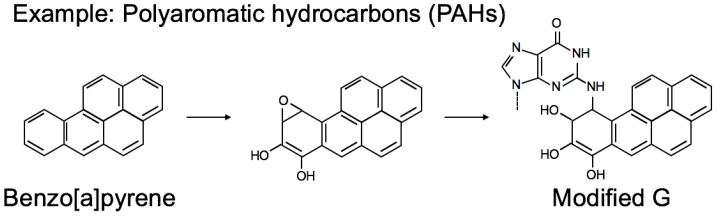
4. Pyrimidine dimers

- UV light causes formation of ring between adjacent pyrimidines
    - Double bonds in adjacent pyrimidines react to form a cyclobuatne ring
    - Most frequently, 2 thymines
- Translesion DNA polymerases required to replicate DNA past pyrimidine dimers
    - But are more error-prone than normal replicative DNA polymerases
    - Increases risk of mutation
- Result:
    - Blocks DNA replication
        - Can be overcome by translesion polymerases

5. Other base modifications

- Ionizing radiation (e.g. gamma rays and x-rays)
  - Causes
- Mutagens react with DNA bases, leading to changes in base-pairing properties or stalled replication
  - Chemical mutations (e.g. $O_2\bullet^-$)
    - First intermediate formed during reduction of oxygen to water by cytochrome oxidase
- Result
  - Mutagenic chemical changes in bases

Example: Polyaromatic hydrocarbons (PAHs)



6. Strand breaks

- Ionizing radiation or mechanical stress can break sugar-phosphate backbone
- Either of one strand (single-strand break) or of both strands (double-strand break)
- Neither normal replicative DNA polymerase nor translesion polymerase can synthesize DNA past break in template
- Double strand breaks can cause chromosomal abnormalities or result in cell death

<u>DNA repair systems</u>

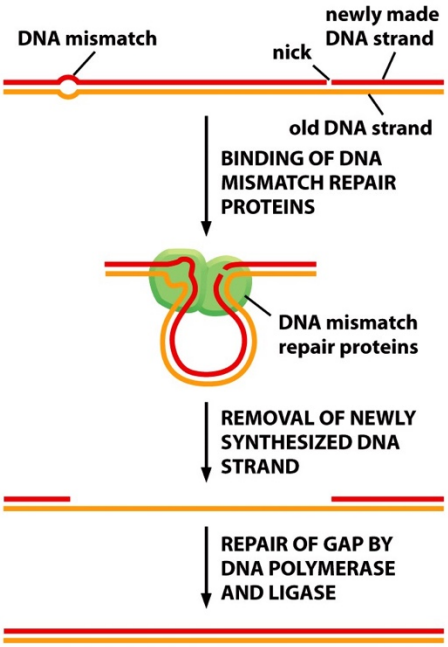
- If single alteration in DNA molecule is left unrepaired, it could interfere with replication process and may cause cell death
- DNA damage can also cause mutations
  - Alter sequence of expressed proteins
  - Impair functions

1. Proofreading during DNA replication

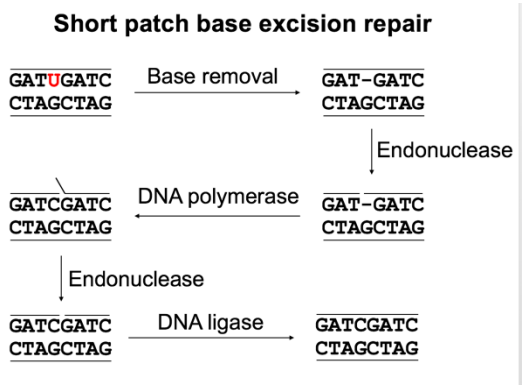
- See topic 20

2. Mismatch-repair

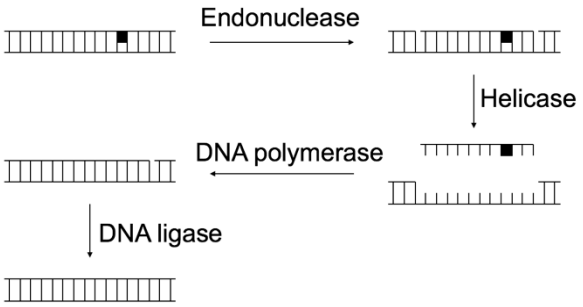
- Correction of mistakes made by DNA polymerase on newly synthesized strand that escaped correction by proofreading
- 99% success rate
- Involves re-synthesis of potentially long stretches of DNA
- After repair machinery has detected site of mismatch, it must determine which strand is the newly synthesized strand
  - In bacteria
    - Scans DNA in both directions until it finds methylated base
    - Most bacteria add methyl groups to bases within specific sequences after replication
    - Unmethylated strands haven't had time to become methylated
      - i.e. unmethylated strand is the newly synthesized strand
- endonuclease nicks backbone of newly synthesized strand
- exonuclease removes bases from nick of mismatch site
  - can be hundreds of nucleotides away
- repair DNA polymerase fills in gap created by exonuclease
- in eukaryotes
  - newly synthesized strand found by searching for intentional nicks (single-stranded breaks) in new strand
  - origin of these nicks is unclear
  - newly synthesized strand removed by exonuclease and resynthesized, as in bacteria



3. **Base excision repair (BER)**
   - Repair of
     - Modified bases
     - Abasic sites
     - Single-strand breaks
   - Steps
     - Damaged base removed (if necessary)
       - Removes base, not nucleotide. Sugar backbone still intact
     - Endonuclease nicks sugar-phosphate backbone (if necessary)
     - Repair DNA polymerase adds 1 (short patch) or 2-10 (long patch) new nucleotides
     - Excess DNA trimmed from damaged strand
     - DNA ligase seals sugar-phosphate backbone



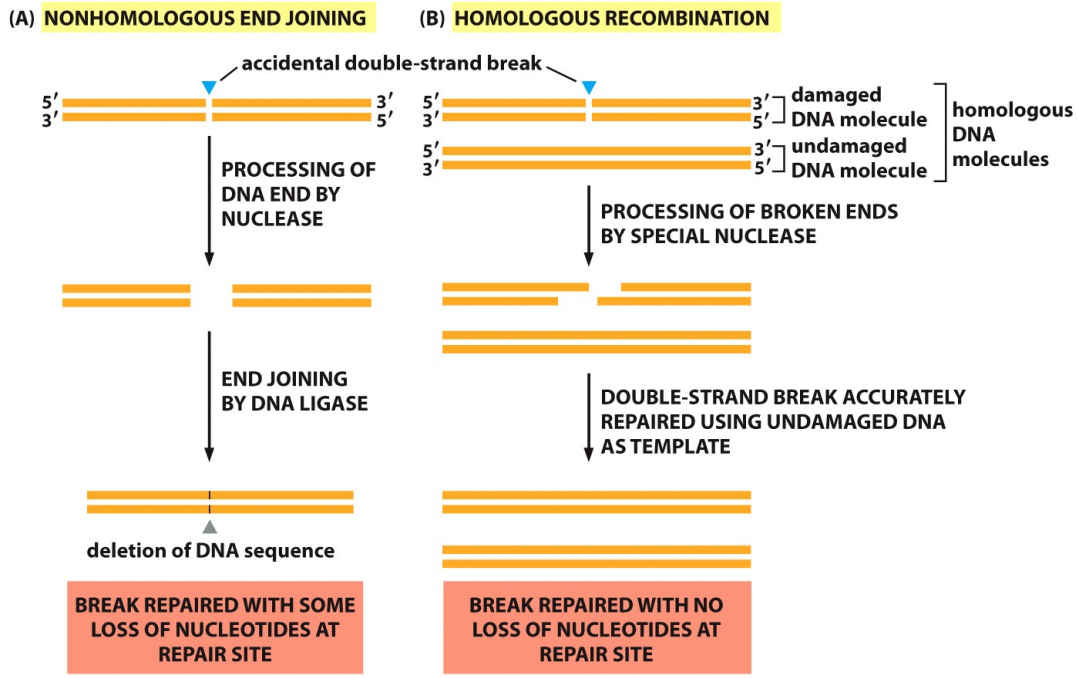
4. **Nucleotide excision repair (NER)**
   - Repair of pyrimidine dimers and damage that disrupts double helix
   - Effects more than one base
   - More disruptive to DNA double helix
   - Cut backbone at either side
   - Nothing to keep DNA there, so basically removes damaged section
   - Steps
     - Endonuclease nicks damaged strand at 2 sites 10-30 bases apart
     - Helicase removes damaged section
     - DNA polymerase re-synthesizes missing section
     - DNA ligase seals sugar-phosphate backbone



5. **Homologous recombination (HR)**
   - One of 2 methods to repair a double-strand break
   - Proteins bind to site of damage and recruit DNA
     - From other copy of the chromosome
     - Complementary to the DNA surrounding the break
   - Complementary DNA used as a template to synthesize new DNA to restore the strand to is original condition
   - Homologous recombination conveniently short after replication when another copy of the chromosome is nearby

6. **Non-homologous end-joining (NHEJ)**
   - Second method to repair double-strand break
   - Ends are made blunt with a nuclease
   - Ends ligated together to form a double-helix
   - Non-homologous end-joining results in loss of DNA relative to sequence that existed before damage
   - Accomplishes repair without need for other DNA

For double strand breaks….



- If you have one, and you try to do replication, chromosomes will become weird
    - They'll become stuck together in weird ways
- Make sure they are fixed before replication and cell division
- 2 strategies:
    - 1 → NHEJ → just take duct take and tape the spine together
        - NHEJ
        - Wanna fix it quickly
        - Process the ends
        - Chewing away some if it needs to
        - Will lose away some nucleotides but it doesn't matter
        - If you're walking done the street and you see a book on the sidewalk
        - You wanna fix the poor book cuz its broken in half
        - Book has no page numbers so you cant just look and see if pages are missing or not
        - Its in a language that you cant understand
    - 2 → If I really care → go to a library and try to find another copy of the same book, compare the characters, and find out if there are any pages missing then seal it up. No info missing
        - HR
        - Will take some time
        - Loop and find on the exact same spot on another chromosome
        - And add the exact same copy
        - If you have 2 different alleles on the same gene, so there are some minor differences on the same strand
        - This is better
        - You have to find the exact other copy of DNA
        - So it takes longer
        - Its accurate tho, but just takes a really long time

Summary

- Our DNA molecules are continuously being damaged through spontaneous and environmentally caused processes
- Damage can cause harmful mutations
- Many DNA repair systems exist, with different mechanisms depending on nature of damage